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THE FAMILIAL OCCURRENCE OF HYPOPLASTIC RIGHT LUNG WITH SYSTEMIC ARTERIAL SUPPLY AND VENOUS DRAINAGE "SCIMITAR SYNDROME"[1]

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The association of a hypoplastic right lung, a "scimitar" shaped shadow of an anomalous pulmonary vein along the right cardiac border and a shift of the heart to the right simulating dextrocardia is now a well recognized clinical syndrome. Park (1) gave one of the earlier descriptions of the anatomic findings. The literature has been reviewed by Halasz, Halloran and Liebow (2) who also made an extensive study of the associated anomalies of the right lung. Recently we have had the opportunity to study a father and daughter who demonstrated this syndrome. The association of anatomical anomalies was similar in both, but produced different clinical and hemodynamic manifestations. The father was entirely asymptomatic whereas severe disability was present in the child. Both were studied with cardiac catheterization, angiocardiography and bronchography. A right pneumonectomy was performed on the child but she did not survive the procedure. The studies performed yielded some new information which may lead to a better understanding of this complex malformation.

Case 1: Howard T. age 30 years (date of birth, April 25, 1927; JHH #768361) attended the Harriet Lane Home Cardiac Clinic with his daughter and gave a history of having been refused for the Army because "his heart was in the right side of his chest." He stated that he was and always had been completely asymptomatic, but later questioning of his mother revealed that he had had many severe respiratory infections as a child and had been hospitalized twice for pneumonia in the first five years of life. He was said to have been frail and underweight as an infant but from about five years of age, was healthy apart from being somewhat thin.

On *examination* he was a thin well-developed man with no dyspnea or chest deformity. Cardiac pulsations were felt to the right side of the sternum although the apex beat was palpable about two centimeters be-

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yond the left sternal edge. Cardiac dullness was present six centimeters to the right and four centimeters to the left of the mid-sternal line. The heart sounds were louder to the left of the sternum and were normal in intensity. The pulmonic second sound was normally split.

Fluoroscopy and x-ray confirmed that the apex was on the left although most of the heart was in the right chest (Plate I Fig. 1). There was no chamber enlargement. The pulmonary vascularity was within normal limits and the aortic arch was on the left. The heart was thought to be dextroposed but otherwise normal at the first examination, and a typical scimitar-shaped shadow beside the right heart border, pathognomonic of drainage of the right pulmonary veins into the inferior vena cava, was overlooked. (The phrase "scimitar syndrome" seems a convenient way of alluding to this condition, all other descriptive titles being unduly cumbersome.) The *electrocardiogram* (Fig. 5) was within normal limits.

After the correct clinical diagnosis had been made on his daughter, Sharon, the father's films were re-examined and he was submitted to further study in 1958 at the age of 31 years. *Cardiac catheterization* (Table I) revealed a localized area in the inferior vena cava just below the diaphragm where the oxygen saturation was 95 per cent. The right ventricular and pulmonary artery pressures were normal. *Bronchography* showed the following. (Report by Dr. D. Torrance): "The bronchi of the right lung are anomalous, there being one large anterior bronchus which supplies the middle and upper lobe areas. There is no well-defined upper lobe bronchus separate from the middle lobe; it is impossible to say whether this corresponds to the absence of the upper or the middle lobe, although from the fact that there are two major divisions of this large bronchus, one might assume that the upper lobe is missing. No diverticula are seen."

Angiocardiography (Dr. G. Schultz) confirmed that the scimitar-shaped shadow in the right chest was formed by the right pulmonary veins joining a common trunk to enter the inferior vena cava below the diaphragm. The right and left pulmonary arteries appeared equal and normal in size. No anomalous arteries to the right lung could be identified.

In view of his excellent clinical condition, no treatment was considered indicated.

Case 2: Sharon T. (date of birth, October 8, 1955; Harriet Lane Home #B-30991) was referred to the Cardiac Clinic of the Harriet Lane Home by Dr. Harold Bix of Baltimore. She was followed from eight months of age until her death at two and a half years.

Family History: Her mother was 39 years and her father 28 years old at the time of her birth. There was one younger female full sibling born in 1957, who was examined and found to be normal. Her mother was also

TABLE I

Catheterization Data (March 1958)

	Case 1: Howard T. 31 years				Case 2: Sharon T. 2½ years			
	O		Pressures mm. Hg.		O_2		Pressures mm. Hg.	
	Vol. %	Sat. %	Instant	Mean	Vol. %	Sat. %	Instant	Mean
SVC	11.9	65			9.6	64		
IVC								
1	11.7	64			10.1	67		
2	12.7	69			10.1	67		
3	17.4	95						
RA								
high	11.9	61			10.2	68		
mid	13.0	71		2	10.4	69		
low	13.0	71						
RV								
in	13.0	71	16/0	4				
mid					10.4	69		
out	12.3	67			10.4	69	85/6	36
MPA					10.7	71	85/50	72
LPA	12.5	68	14/3	6	10.7	71		
FA					13.8	92	93/41	65
LP wedge								2
Capacity	18.35				14.99			

examined and was normal; she had had two children, one male and one female, by a previous marriage: both these children were now in their twenties and were said to be perfectly healthy. They were not examined. There were three normal grandchildren by this first marriage.

Sharon's father, Howard T., is reported above as Case 1. He had two male siblings, both normal, who have each had one normal child. Sharon's paternal grandmother was examined at 55 years of age and was normal; her paternal grandfather died at 40 years of age following a cerebral operation: he had had an operation on his right lung at about 20 years of age. We had at first understood that this was a right pneumonectomy but a little historical discussion revealed that the operation antedated by several years the first successful pneumonectomy. From the severe ensuing thoracic deformity, the large scar and the occasional recurrent drainage from a sinus in the right chest, all of which were described

graphically by his widow, we deduced that this operation had been for drainage of a right-sided empyema. It is interesting to postulate, but impossible to prove, that this man also had an abnormal right lung with pneumonia and empyema as a complication and that the malformation was inherited by his oldest son and his elder granddaughter.

Past History: Sharon was born at full term weighting six pounds, twelve ounces following a pregnancy complicated by a very mild degree of toxemia. She was thought to be normal at birth and made good progress until seven months of age when she was hospitalized for the first of many attacks of "pneumonia". During this and subsequent similar attacks, there was high fever, vomiting, wheezing respirations and generalized rales in the lungs. On one or two occasions, the physical signs were more marked in the right lung but this was inconstant and a definite area of consolidation was never found. During this first illness, she was hospitalized for one week and a cardiac murmur and cardiac enlargement were detected.

Physical Examination at eight months revealed a moderately well-nourished infant weighing 7.2 kilograms. The peripheral pulses were full, the blood pressure in the arm was 95/40. The heart was markedly enlarged and occupied most of the right chest. The apex was not definitely localized and no thrill was felt. The second sound was loud, single and palpable and was maximal just to the right of the sternum. There was a grade 2 systolic murmur maximal in the second right interspace continuing across the second sound into early diastole. The systolic component of the murmur was transmitted over the right chest and to the back. The liver was palpable one centimeter below the right costal margin. On subsequent examinations, the cardiac signs showed little change although the diastolic murmur was not consistently present.

Fluoroscopy and x-ray showed a large heart which almost filled the right chest cavity, with a cardio-thoracic ratio of about 58 per cent (Plate I Fig. 2). In the oblique views both anterior and posterior ventricles appeared enlarged. The pulmonary vascularity was thought to be within normal limits although the left pulmonary artery appeared large: the right could not be identified. Barium swallow showed a left aortic arch and slight left atrial enlargement.

Electrocardiogram (Fig. 5) showed right axis deviation and severe right ventricular hypertrophy. The P waves were inverted in Lead I and upright in AVR which was thought to confirm the clinical impression of dextrocardia.

Clinical Course: She gained weight poorly, weighing about ten kilograms at two and a half years. Her height also remained at about the third percentile for her age (85 centimeters at two and a half years). She

presented a severe feeding problem throughout. She sat up at six months and walked and talked at about one year. She had febrile episodes associated with a cough and wheezing respirations about every six weeks from eighteen months to two and a half years. Most attacks lasted several days and required therapy, and several required hospitalization. There was definite reduction of exercise tolerance and she would often lie down to rest while playing. Digitalis was given from eighteen months of age. She was never cyanotic and the hemoglobin and hematocrit remained normal.

The original clinical diagnosis was isolated dextrocardia with patent ductus and probably another intracardiac lesion. The presence of severe pulmonary hypertension was recognized from an early age. Because of the poor progress and recurrent chest infections, further study was considered indicated.

Bronchography showed the following. (Report by Dr. D. Torrance): "The bronchi of the right lung are sparse and abnormal in appearance. One large branch supplying apparently the superior and inferior portion of the lung anteriorly has a markedly anomalous distribution. It does not correspond to a normal middle lobe because of the large superior portion of the lung it supplies. In addition, there is a small bronchus which would correspond normally to the right upper lobe, but which evidently supplies appreciably less than in the normal instance. The right lung appears markedly hypoplastic with the hypoplasia most marked in the upper lobe."

Cardiac catheterization confirmed the presence of severe pulmonary hypertension. No definite left to right shunt was demonstrated. The right pulmonary artery was not entered. The right heart chambers were displaced into the right chest but bore a normal relation to each other. Table I gives detailed catheterization data on Sharon and her father.

Angiocardiography (Dr. G. Schultz) was performed by injecting twelve milliliters of 70 per cent Urokon through a Number 7 Lehman catheter in the right ventricle (Plate I, Figs. 3 and 4). This study revealed the correct diagnosis. The marked cardiac displacement to the right was confirmed. The main and left pulmonary arteries were markedly enlarged, but the right pulmonary artery was very small, only about one quarter the size of the left. The right pulmonary veins were seen to form a trunk draining below the diaphragm. This scimitar-shaped trunk lay behind the cardiac shadow and was therefore not seen on the regular chest x-ray. The left pulmonary veins entered the left atrium normally. The left ventricle and aorta appeared normal and the aorta descended on the left.

Following angiocardiography and the recognition of the correct diagnosis, it was decided that the child might benefit from a right pneumonectomy. The factors influencing this decision are analyzed in the discussion.

On March 25, 1958 a right thoracotomy was performed under cyclopropane anesthesia. The right lung was composed of an upper, middle, and lower lobe. The entire pulmonary venous drainage entered a single large vein which swept inferiorly and passed through the diaphragm near the site of entry of the inferior vena cava into the chest. The right pulmonary artery was smaller than normal and divided into two branches which supplied the two segments of the middle lobe. One large systemic artery and several small ones were found to course through the diaphragm and enter the inferior surface of the right lower lobe. Another systemic artery from the upper mediastinum which was seen to enter the lung substance was divided. The course of this vessel in the lung could not be identified in the surgical specimen. Figure 6 illustrates the findings at operation.

The pressure in the pulmonary artery was recorded at 127/63 mm. Hg while the systemic pressure was 100/75 mm. Hg. The mean pressure in the right pulmonary vein was 18 mm. Hg and in the inferior vena cava 8 mm. Hg. The vessels were individually isolated, ligated and divided and a pneumonectomy was performed. The child died a few hours after the procedure.

Postmortem Examination (Autopsy No. 28053) showed that there was slight mediastinal emphysema and a shift of the heart and mediastinum to the right. Surgical removal of the right lung had left a smooth pleural cavity in which the vessels which perforated the right leaf of the diaphragm appeared to be the only abnormal finding. After removing ligatures one could pass a probe through the largest vessel into the inferior vena cava, and microscopic examination confirmed that this large vessel was a vein. No evidence of obstruction to this vessel could be found. The smaller vessels which perforated the diaphragm were found to arise from the abdominal aorta above the coeliac axis and these presumably were aberrant branches of the phrenic arteries.

The external configuration of the heart showed a prominent right ventricle. The venae cavae entered the dilated right atrium in a normal manner. No thrombus material was present in the atrium or its appendage. The foramen ovale was sealed. The right ventricle was hypertrophied and dilated and the myocardium measured about 1.0 centimeter in thickness. The pulmonary conus was hypertrophied and the endocardium in this area appeared opaque. The pulmonary valve leaflets were delicate. The pulmonary artery was greatly dilated but no atheromatous plaques were present in the major branches. The right branch of the pulmonary artery had a substantially smaller lumen than the left. It had been ligated before it divided to supply the lobes of the right lung.

Examination of the *left lung* showed that the very dilated left pulmo-

nary artery gave off branches to all lobes in a normal manner. A small patent ductus arteriosus (lumen of 2 mm. diameter) communicated with the aorta, and a small ridge in the aorta just proximal to the ductus orifice appeared to narrow the aortic lumen slightly.

The pulmonary veins from the left lung entered the left atrium in a normal manner, whereas no pulmonary venous drainage to the left atrium from the right lung could be identified. The left ventricle was slightly hypertrophied and the aortic valve was bicuspid.

Examination of the *right lung*, which had been removed at operation, showed three easily distinguishable lobes as illustrated in the diagram of Figure 6. Perfusion of the afferent and efferent vessels with radiopaque material established that the small right pulmonary artery supplied the small ($2 \times 2 \times 3$ cm.) upper and somewhat larger ($5 \times 3 \times 2$ cm.) middle lobes of the right lung (Plate II, Fig. 7). Injection of the largest anomalous artery, which arose from the aorta below the diaphragm, showed that this vessel supplied the lower and largest lobe ($8 \times 7 \times 3$ cm.) of the right lung but did not supply any other portions of the right lung (Plate II Fig. 9).

A congenital malformation of the urinary tract was also present. On the right, the kidney was lobulated and separate ureters arose from the upper and lower poles to join a short distance from the pelvis of the kidney. On the left there was a complete duplication of the ureter.

Microscopic findings: Sections from both lungs and from the aorta and main pulmonary artery were stained with Verhoeff-Van Gieson elastic tissue stain and also with hematoxylin and eosin. Pulmonary vascular changes were present in an unexpected distribution: they were minimal in the right upper and middle lobes, which were supplied by the small right pulmonary artery, moderately severe in the right lower lobe which was supplied by systemic vessels, and most severe in the left, or "normal" lung.

The pulmonary arterial bed of the right upper and middle lobes appeared essentially normal. The medium sized and small arteries showed a well-defined muscular media but this did not exceed the thickness which is normal at this age. The vascular lumens were wide. Very rare small patches of intimal thickening were found.

The intrapulmonary arteries of the right lower lobe (that supplied by the systemic circulation) showed thick muscular walls and narrowed lumens (Plate III Fig. 10). Intimal thickening was frequently seen, especially at the branching of small vessels (Plate III Fig. 11), and occasionally it occluded the arterial lumen. These changes in the intima and the thickening of the muscular wall correspond to pulmonary hypertensive changes of grades 1 to 3 in the classification of Heath and Edwards (3).

In the left lung pulmonary hypertensive changes were even more severe. Medial hypertrophy was marked and intimal fibrosis of the small pulmonary arteries frequently resulted in obliteration of the arterial lumens. (Plate III Fig. 12). In several areas small pulmonary arteries and their arteriolar branches contained proliferating cellular endothelial tissue and presented the appearance of "plexiform" lesions. Thus this lung, with a normal arterial supply and venous drainage, showed vascular changes corresponding to grades 2 to 4, changes of severity rarely seen in so young a child.

The pulmonary veins in the right upper lobe, the right middle lobe and in the left lung were thin walled and appeared normal. The pulmonary veins of the right lower lobe showed some thickening of their walls due to an increase in the number of collagen and elastic fibres and in subendothelial smooth muscle fibres. This change was present in the larger veins and extended down to the smallest branches.

Sections from the *main pulmonary artery* demonstrated some variations in the pattern of the elastic lamellae, but for the most part the elastic fibres were arranged in regular continuous layers similar to the usual aortic pattern. Dr. Jesse Edwards examined the sections and stated that he believed the pulmonary arterial media possessed an aortic pattern, indicating that the pulmonary hypertension had been present from birth.

Evidence of *chronic bronchopulmonary* infection was found in the left lung where the bronchioles showed thickened walls and foci of inflammatory cells. No such alterations were present in the right lung. In the right lower lobe, however, a parenchymal abnormality may have been present: many bronchioles terminated in wide spaces which gave the impression of cystic dilatation, but the lining epithelium was delicate. It has been suggested that these spaces were artefacts caused by the perfusion studies, but the possibility of a malformation of the pulmonary parenchyma cannot be excluded with certainty.

DISCUSSION

Familial Incidence

The occurrence of so rare a lesion in both father and daughter can only be accounted for by direct inheritance. Our present knowledge of the genetics of congenital heart disease is still scanty, although families have been recorded with the same malformation occurring in three generations (4, 5). It seems probable that more knowledge will be gained in the future by the study of malformations in successive generations rather than by further analysis of the siblings of affected children.

Embryology

Unlike all the other types of anomalies of the pulmonary venous return, this condition is accompanied by abnormalities of the right lung of varying degrees of severity. It should therefore be considered as fundamentally an abnormal development of the right lung bud and as representing a more primitive type of malformation than the usual anomalies of the pulmonary venous return which occur between Horizon XIV and XV of embryonic life (6).

This malformation of the lung in some respects resembles that described by Pryce as intralobar sequestration (7, 8). In sequestration the anomalous lung or lobe has systemic arterial blood supply and its venous connections may be with derivatives of the cardinal venous system; the bronchial tree usually does not connect with the affected lobe, which contains cystic areas of sequestrated bronchial epithelium.

In the "scimitar syndrome" the venous connections of all lobes of the right lung are anomalous whereas the systemic arteries supply only part of the lung, usually the right lower lobe: also the lung connects with the right main bronchus. No definite cysts have been reported although bronchial diverticula were described by Halasz (2); and cyst-like areas were present in the right lower lobe in our Case 2. In future cases a careful search should be made for such cysts.

Hypoplasia of the right lung (9, 10, 11, 12) is an integral part of the "scimitar syndrome." Although partial systemic arterial supply from below the diaphragm may occur in patients with hypoplasia of the left lung we have found no case of a left sided "scimitar syndrome," possibly because of the extreme rarity of a left sided inferior vena cava. Hemivertebrae have been described in association with hypoplasia of the lung (11, 12) and have been observed in other cases of the "scimitar syndrome" (13) but were absent in the patients described here.

The malformation should thus be thought of as a right sided cardiopulmonary malformation rather than as one of the more usual types of partial anomaly of the pulmonary venous return related to defects in atrial septation (14).

Clinical Course

The reported cases form two distinct groups, well exemplified by the two patients here presented. In one group, represented by Case 1 (or even better by McKusick's case (15) a Negro female of 26 years who had had six normal pregnancies before being studied), the condition is benign and is detected only when x-ray or clinical examination reveal dextroposition of the heart and a scimitar shadow. These cases have no pulmonary

hypertension and the degree of cardiac displacement is slight and indeed, is frequently not commented upon. The clinical diagnosis is clear if the scimitar shadow on the x-ray is once recognized.

In the second group, exemplified by our Case 2, and also described by Park (1) and Halasz (2), there is a gross degree of dextroposition of the heart, numerous severe respiratory infections, and in our case at least, severe pulmonary hypertension. These patients with severe hypoplasia of the right lung may be mistakenly thought to have dextrocardia: angiocardiography readily demonstrates the abnormal right pulmonary vein.

Physiopathology

The literature was studied to find whether all the benign cases have a normal pulmonary artery pressure as does our Case 1 and all the severely affected have pulmonary hypertension, as did our Case 2. The cathererization findings on a total of fourteen cases are summarized in Table II.

The majority of these patients were adults, many were asymptomatic and in most instances the pulmonary artery pressure was normal. Halasz (2) reports "pulmonary hypertension" in a 4 year old boy but gives no details. Our Case 2 appears to be unique in the severity of her pulmonary hypertension. The following were considered as possible etiologic factors:

1. *Chronic infective changes in the lung* causing cor pulmonale: no evidence for such a mechanism was found at autopsy. The inflammatory changes present were confined to the bronchial tree and there was no destruction of pulmonary parenchyma.

2. *Systemic arterial blood supply to the right lower lobe* was considered but there was no communication with the pulmonary vascular tree and similar vessels are found in the asymptomatic patients without pulmonary hypertension.

3. *Increased pulmonary blood flow* due to the anomalous pulmonary venous drainage could result in "dynamic" pulmonary hypertension (26). However, flow through the hypoplastic lung of our patient was small and no left-to-right shunt could be calculated. In other cases in whom a left-to-right shunt was present, the pulmonary artery pressure was usually normal. The magnitude of the shunt was, in most instances, considerably less than would be expected if the blood flow to the right and left lungs were of normal proportions (27), suggesting that in this malformation the major portion of the cardiac output is directed to the left lung, possibly due to the small size of the right pulmonary artery.

4. *Unilateral pulmonary venous obstruction* may produce pulmonary hypertension (28, 29, 30). The pressure in the right pulmonary vein in

TABLE II

Drainage of Right Pulmonary Veins into IVC Catheterization Findings

Reference	Age	Sex	Relation of RPV to Diaphragm	Assoc. Defect	Pressure mm. Hg				RPV O_2 Sat. %	L-R Shunt % of Pulm. Flow	Hypoplasia Rt. Lung*
					RPV	RA or IVC	RV	PA			
Case 1 H.T.	31	M	below	—	cath. 18 surg. 18/8	2	16/0	14/3	95†	None	+
Case 2 S.T.	$21\frac{1}{2}$	F	below	PDA		8	85/6	85/50 127/63		None	++
Kaplan (16)	6	M	below			2	26/3	25/10	100	None	
Dotter (17)	41	M	below		Not Stated					Present	+
Cooke (18)	20	M	below		4–6	2–6			98	30%	–
Snellen (19)	21	F	above		5	6	30/−3 50/20	40/20	97	30%	–
Mankin (20)	43	F	?					29/12	96†	30%	+
Arvidsson (21)	37	M	?					13 mean		40%	+
McKusick (15)	32	F	?			8/2	39/3	31/11		20%	+
Kirklin (22)	32	F		ASD	Not Stated					50%	–
Halasz (2)	4	M		intra-cardiac L-R shunt	"Pulmonary Hypertension"						–
Wood (23)	26	M	above	ASD			27/0/−5	27/10		50%‡	–
Steinberg (24)	55	F	?			8	34/8			30%	–
Loogan (25)	56	F	below			5/8	27/0	25/12	98	27%	–

* Cases with shift of mediastinum

† In IVC opposite PV

‡ Shunt at atrial level, no shunt due to RPV

our Case 2 was higher than that in the inferior vena cava although no anatomical obstruction to flow could be found. The possibility remains of physiological obstruction to a pulmonary vein which passes through the diaphragm. Some thickening of the veins of the right lower lobe was also compatible with venous obstruction: the absence of such changes in the right upper and middle lobes may be explained by the small arterial inflow into these lobes. However, in Kaplan's case (16) a similar pressure gradient between the anomalous vein and the inferior vena cava was not associated with pulmonary hypertension.

5. *Hypoplasia of the right lung* appears important in determining the clinical course. Its possible relation to the development of pulmonary hypertension is less clear. In a patient with a normal heart hypoplasia or even agenesis of one lung is well tolerated (10), and although Warner (31) has suggested that early signs of cor pulmonale may occasionally be present, we have found no instance of proven pulmonary hypertension. It may be that in the "scimitar syndrome" the hemodynamic alterations are well tolerated in the majority of patients with only mild or moderate pulmonary hypoplasia, but that progressive pulmonary hypertension develops if the hypoplasia is severe. The pulmonary hypertension present in Case 2 did not appear due to any of the mechanisms discussed above, and it seemed likely vasomotor causes were responsible. This is also suggested by the presence of "injury" changes in the vessels of the left lung. The physiologic data indicate that the major part of the cardiac output was directed into the left lung. This lung, if normal, should have been able to carry this increase in blood flow without any rise in pulmonary pressure (32): if, however, the larger volume of blood were ejected against an increased resistance, injury changes could result. The unusually severe vascular changes suggest that a high resistance was present from an early age, and the "aortic pattern" of the elastic tissue of the main pulmonary artery also indicates pulmonary hypertension dating from birth (33). The absence of similar changes in the upper and middle lobes of the right lung may indicate that the flow through the small right pulmonary artery protected those vessels from injury.

Treatment

Pneumonectomy was undertaken in the hope that if the right lung were the site of the numerous respiratory infections, and also responsible for the severe pulmonary hypertension, its removal might benefit the child and cause a drop in the pulmonary artery pressure. Autopsy showed that this hope was not justified. The left lung was already the seat of such severe pulmonary vascular changes that no further increase in blood flow could be tolerated.

It would seem that the role of surgery in this malformation is limited. The benign cases with little or no pulmonary hypertension probably do not require treatment. Severe pulmonary hypertension, as in our Case 2, appears to be a contraindication to operation. The only cases where pneumonectomy might be of value are those with severe infective changes in the right lung. In patients with a large left-to-right shunt, transplantation of the anomalous vein to the left atrium could be considered. A large left-to-right shunt seems usually to be associated with an additional intracardiac defect, which would best be corrected at the same operation.

SUMMARY

A father and daughter are presented who had an identical anatomical malformation of the right lung: the right lung was smaller than normal, the right pulmonary venous return was into the inferior vena cava and there was systemic arterial supply to the right lower lobe. The father had a mild degree of dextroposition of the heart and pursued an asymptomatic course without pulmonary hypertension. The daughter had a gross degree of dextroposition of the heart with marked hypoplasia of the right lung and right pulmonary artery: she had frequent respiratory infections, severe pulmonary hypertension and died following right pneumonectomy.

A review of similar cases in the literature revealed that this malformation is usually benign, as it was in the father, but rarely symptoms are present from an early age. The clinical course seems more severe in those with marked hypoplasia of the right lung.

Severe pulmonary hypertension, as observed in the child, has not previously been reported. Microscopic studies revealed pulmonary hypertensive lesions of unusual severity in the small arteries of the left, otherwise normal, lung. These changes were not seen in those parts of the right lung supplied by the right pulmonary artery. The data available suggest the pulmonary hypertension was of vasomotor origin, related either to the extreme degree of hypoplasia of the right lung and its artery or possibly to obstruction of the anomalous pulmonary vein.

Surgery for this malformation appears of limited value.

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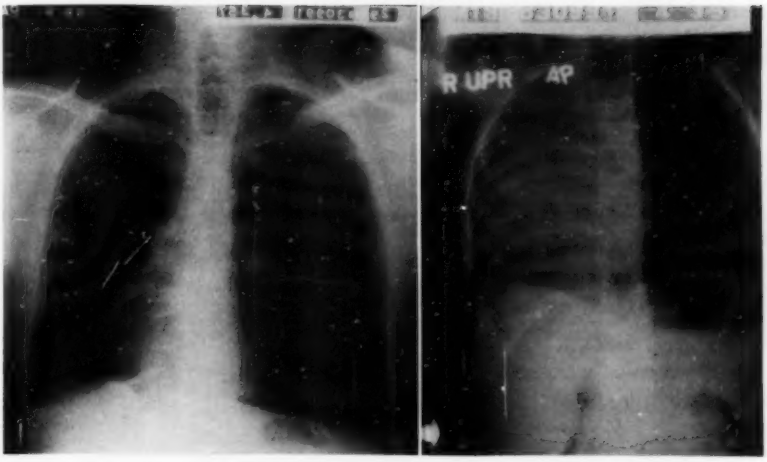
PLATE I

FIG. 1. Upright roentgenogram (PA) of Case 1 showing slight dextroposition of heart. Arrow shows "scimitar shadow" of pulmonary vein.

FIG. 2. Upright roentgenogram (AP) of Case 2 showing gross dextroposition of heart with cardiac enlargement.

FIG. 3. Case 2: angiocardiogram showing opacification of right ventricle, large main and left pulmonary artery. Small right pulmonary artery outlined by dotted line.

FIG. 4. Case 2: angiocardiogram (late) showing dye in small left atrium and aorta. Arrow shows anomalous pulmonary vein draining right lung.


R UPR AP

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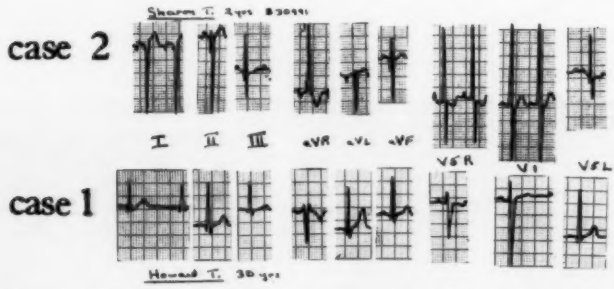

FIG. 5. Electrocardiogram: Case 1 within normal limits. Case 2 marked right axis deviation with inverted P waves in lead 1.

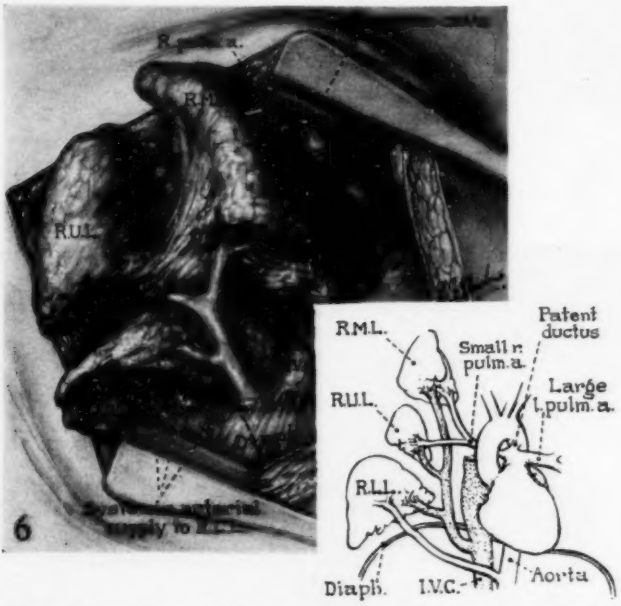

FIG. 6. Drawing of anomalous right lung as seen at operation. The schematic drawing illustrates the described anomalous arterial supply and venous drainage of the right lung. The right middle lobe has been reflected upwards to expose right pulmonary vein.

PLATE II

FIG. 7. Roentgenograms following injection of right lung with contrast substance. Injection of right pulmonary artery showing filling of upper and middle lobes only.

FIG. 8. Injection of anomalous artery from abdominal aorta to right lower lobe.

FIG. 9. Injection of anomalous right common pulmonary vein showing that it drains all lobes of the right lung.

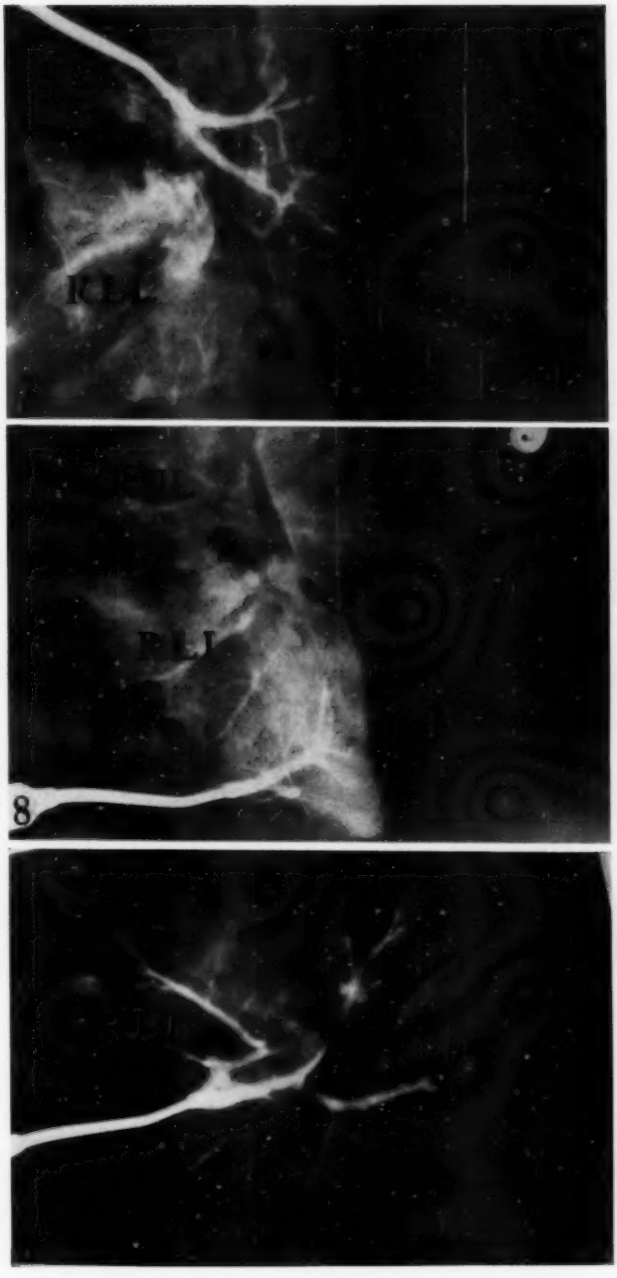
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PLATE III

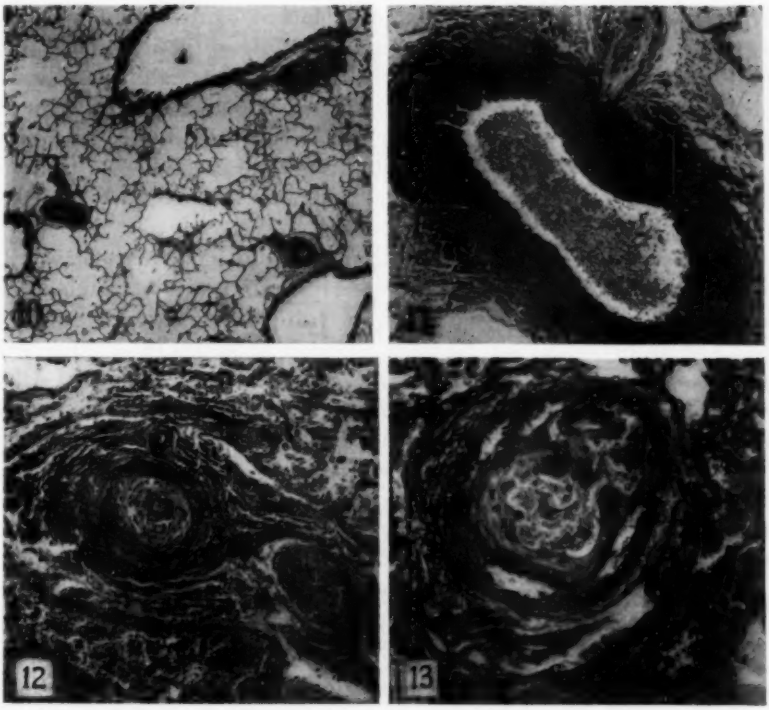
Microscopic sections of the lungs stained with Verhoeff-Van Gieson elastic tissue stain.

FIG. 10. This micrograph (×30) of a section from the right lower lobe (the lobe which is supplied by the anomalous systemic artery and drained by the anomalous vein) shows dilated bronchi and three small arteries with moderately thick muscular walls.

FIG. 11. This light micrograph at higher magnification (×100) of another section from the right lower lobe shows a small artery with proliferation of the intima where the larger vessel gives off a branch.

FIG. 12. This light micrograph (×100) of a section from the left lung (anatomically normal arterial supply and venous drainage) shows a small pulmonary artery with medial hypertrophy and intimal proliferation which occludes the lumen.

FIG. 13. This light micrograph (×200) of another section from the left lung shows intimal proliferation in an arteriolar branch with dilatation ("plexiform lesion").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PATHOGENESIS OF FEVER IN JOINT DISEASE. THE EFFECT OF INTRAARTICULAR PRESSURE AND INTRA-ARTICULAR BACTERIAL PYROGEN[1]

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Infection of the joint is usually accompanied by a marked febrile response in man and in experimental animals. One of the unusual features of this infection is its occurrence in an area with limited ability to expand. Therefore, inflammation is likely to result in an increase in intraarticular pressure (1). Another unusual feature of infection of a joint is the rapidity with which defervescence occurs following aspiration, as illustrated in Figure 1. This defervescence is not necessarily associated with control of the infection.

The defervescence following drainage of an infected joint may be attributed to removal of pyrogenic bacteria and bacterial products, removal of the products of tissue injury, or reduction of intraarticular pressure. The ability of certain bacteria or bacterial toxins to produce fever has been adequately demonstrated (2, 3). In addition, the studies of Atkins and Wood (4) and Bennett and Beeson (5) have implicated the injured neutrophile as a source of endogenous pyrogen. The diffusion of pyrogens out of the joint space could be facilitated by increased synovial permeability to these substances associated with inflammation and/or increased intraarticular pressure. For example, permeability has been found to be increased in inflamed joints (1).

To evaluate the role of pressure in the production of fever in arthritis, the following studies were done.

MATERIALS AND METHODS

Animals: Normal male rabbits of mixed breed and color, weighing 3 to 5 kg. were restrained on an animal board in the prone position for all experiments.

Pyrogen: Purified pyrogen (endotoxin) from *Shigella Flexneri* (6) was prepared in a concentration of 1 mcg. per ml. in 0.85 per cent NaCl.

Serum: Rabbits were bled by cardiac puncture using sterile precautions. The serum was separated after centrifugation and stored at 4° C.

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[2] Work performed while a student at the Johns Hopkins University School of Medicine.

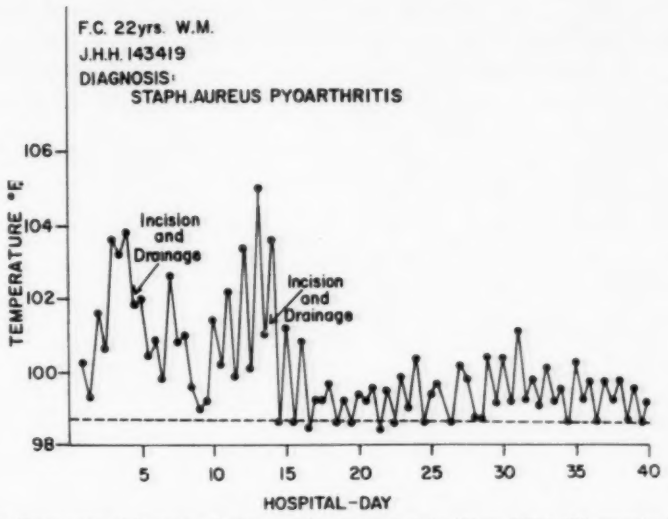


FIG. 1. Response of fever to drainage of a pyoarthritis: 22 yr. WM. Diagnosis: Chronic staphylococcal osteomyelitis left femur and acute pyoarthritis left knee. The prompt defervescence following incision and drainage of the pyoarthritis is illustrated.

Measurement of pressure: An 18 gauge needle was inserted into the left knee of the rabbit and connected to Statham Physiological Pressure Transducer, Model P23Db, by flexible tubing. The pressures were recorded by a Sanborn Twin Viso recorder.

Measurement of temperature: Rectal temperatures were measured at 30 minute intervals with a centigrade thermometer.

EXPERIMENTAL RESULTS

The intraarticular pressure measured by insertion of a needle into the sterile joint of a rabbit is essentially zero. If the needle is allowed to remain in the joint for a period of one or two hours the intraarticular pressure will rise to about 10 cm. of water and remain constant at this level. Intraarticular pressure can be increased to above 20 cm. of water and maintained at this level for at least four hours by intermittent injection of rabbit serum, glycerine or mineral oil. Repeated injections are required to maintain the increased intraarticular pressure, and there are individual variations in the volume of fluid required for maintenance of elevated intraarticular pressure.

An animal restrained in the manner used in these experiments will show a rise in temperature of 0.3 to 0.4° C. over a period of 4 hours, even if there is no further manipulation of the joint.

To determine if increased joint pressure alone is capable of producing fever the following experiment was done.

Three rabbits were restrained as described in Methods, and the intra-articular pressure increased with normal serum, and maintained above 20 cm. of water for 4 hours. A small rise in temperature occurred, but was no different than that observed when joint pressure was not increased. It was, therefore, concluded that increased intraarticular pressure alone is incapable of causing fever in the rabbit under the conditions of these experiments (Fig. 2 and Table I).

To see if bacterial pyrogen instilled in a normal rabbit's joint is capable of causing fever, the following experiment was done.

Two rabbits were restrained as described above, and 0.2 mcg. (0.2 ml.) of bacterial pyrogen was injected into the left knee of each animal. The intraarticular pressure and rectal temperatures were then recorded for 4 hours. The small increase in temperature that occurred was no higher

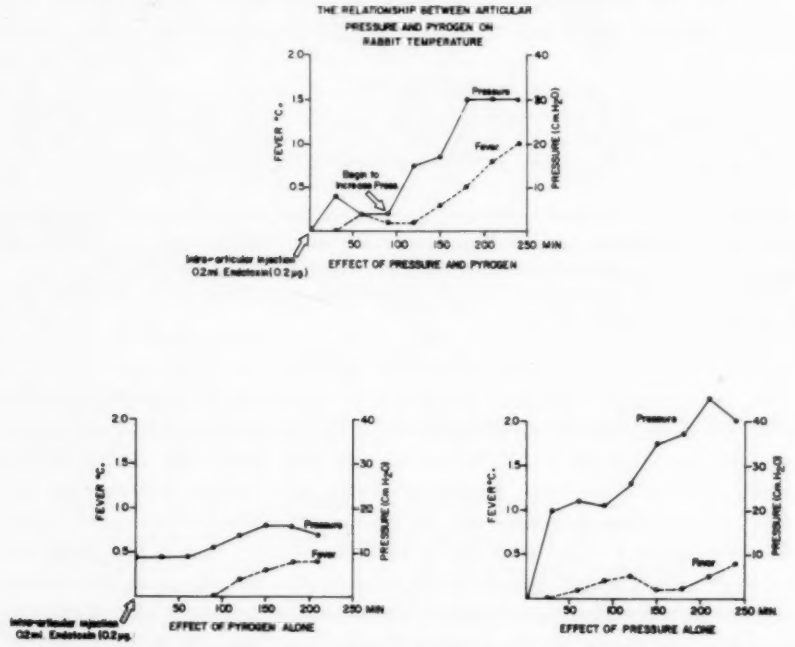

THE RELATIONSHIP BETWEEN ARTICULAR PRESSURE AND PYROGEN ON RABBIT TEMPERATURE
FEVER °C.
PRESSURE (Cm H2O)
Pressure
Fever
Begin to Increase Press.
50 100 150 200 250 MIN
EFFECT OF PRESSURE AND PYROGEN
EFFECT OF PYROGEN ALONE
EFFECT OF PRESSURE ALONE

FIG. 2. Effect of Intraarticular pressure and intraarticular bacterial pyrogen on fever in respresentative animals: 1. Effect of pressure alone—showing no appreciable influence of intraarticular pressure upon rabbit temperature. 2. Effect of pyrogen alone—showing no appreciable influence of intraarticular pyrogen upon rabbit temperature. 3. Effect of intraarticular pressure and intraarticular pyrogen—showing a greater than 1.0°C. rise in rabbit temperature. Joint pressure increased by repeated injection of normal rabbit serum.

TABLE I

Effect of Joint Pressure Alone on Temperature

Rabbit No.	Maximum Temperature Elevation	Average Pressure (cm. saline)
714	0.4°C.	30.7
969	0.45°C.	19.7
570	0.3°C.	45.4
Average	0.38°C.	31.9

TABLE II

Effect of Intraarticular Pyrogen Alone on Temperature

Rabbit No.	Maximum Temperature Elevation	Average Pressure (cm. saline)
505	0.5°C.	7
493	0.4°C.	12
Average	0.45°C.	9.5

than that found in the preceding experiment, and the intraarticular pressure did not exceed 12 cm. of water. (Fig. 2 and Table II). It was, therefore, concluded that bacterial pyrogen in the joint was not capable of causing fever under the conditions of the experiments.

In the preceding experiments, increased intraarticular pressure and injection of bacterial pyrogen into the knee joint were incapable of producing fever. The following experiment was done to investigate the possibility that the inoculation of bacterial pyrogen into a normal joint will result in fever if the intraarticular pressure is elevated at the same time.

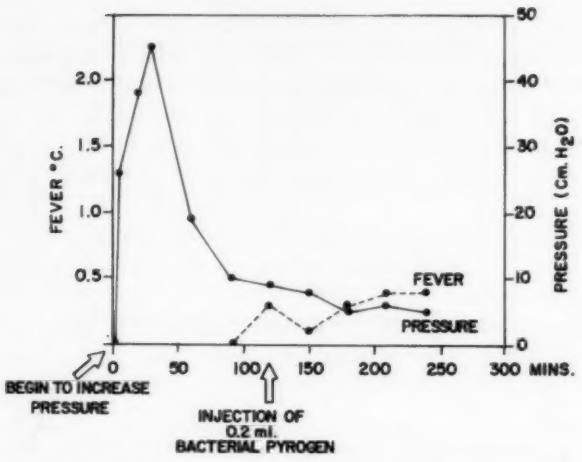
Six animals were restrained in the same manner as described before. In three animals the intraarticular pressure was increased and maintained above 20 cm. of water for 4 hours. As the pressure was rising, 0.2 ml. of pyrogen was instilled into the joint space. Temperatures were then re-

TABLE III

Effect of Increased Intraarticular Pressure and Intraarticular Pyrogen on Temperature

Rabbit No.	Maximum Temperature Elevation	Average Pressure (cm. saline)
572	2.3°C.	39.3
502	1.2°C.	28.3
503	0.7°C.	33.2
Average	1.3°C.	33.8

Note: In these experiments the intraarticular pressure was increased as soon as the pyrogen was instilled into the joint.



EFFECT OF PYROGEN ON A JOINT WHICH HAD PREVIOUSLY BEEN SUBJECTED TO INCREASED INTRA-ARTICULAR PRESSURE

FIG. 3. Temperature response to injection of bacterial pyrogen into a joint which had been previously subjected to an increased intraarticular pressure: The intraarticular pressure was increased at time 0 and maintained above 20 cm. of water for 1 hour. For the next hour the pressure was allowed to fall. Bacterial pyrogen was injected when the pressure had returned towards normal and the temperature rise was insignificant.

corded for 4 hours. In the remaining three animals, 0.2 ml. of pyrogen was instilled in the joint space, and after 90 minutes the intraarticular pressure was increased and maintained above 20 cm. of water for 150 minutes.

In both groups of rabbits the temperature elevations were much higher than those found in the preceding experiments. The temperature in the second group of animals, moreover, did not begin to rise until the pressure was increased (Fig. 2 and Table III). It was, therefore, concluded that

TABLE IV

Effect of Increased Intraarticular Pressure and Intraarticular Pyrogen on Temperature

Rabbit No.	Maximum Temperature Elevation	Average Pressure (cm. saline)
573	1.3°C.	31.7
507	0.8°C.	22.0
509	1.0°C.	26.4
Average	1.1°C.	26.3

Note: In these experiments the intraarticular pressure was not increased until 90 minutes following the injection of bacterial pyrogen into the joint.

TABLE V

Effect of Pyrogen and Prior Increase of Joint Pressure on Temperature

Rabbit No.	Maximum Temperature Elevation	Average Pressure Before Pyrogen	Average Pressure After Pyrogen
628	0.4°C.	32.7	6.8
824	0.25°C.	20.5	12.1
Average	0.33°C.	26.6	9.5

Note: Intraarticular pressure allowed to return toward normal before pyrogen was injected into the joint.

bacterial pyrogen was capable of inducing fever only when the intraarticular pressure was increased above 20 cm. water.

The preceding experiment showed that intraarticular pressure and intraarticular pyrogen acting together in a normal joint are capable of inducing fever in the rabbit. The possibility that increased intraarticular pressure injured the synovia, allowing the entrance of pyrogen into the circulation was considered, but under these conditions the effect should persist for a period of time after the intraarticular pressure was decreased. To investigate the possibility that the effect of increased intraarticular pressure might persist after the pressure is lowered, the following experiment was done.

Two rabbits were restrained in the usual manner. The intraarticular pressure was raised above 20 cm. of water and maintained for 1 hour. For the subsequent hour the intraarticular pressure was allowed to decrease. Two-tenths ml. of bacterial pyrogen was then injected into the joint and the temperature and pressure recorded for 2½ hours.

Although the intraarticular pressure remained slightly above zero, it was within the range of normal; and there was no significant febrile response (Fig. 3 and Table IV). The intraarticular pressure, therefore, had to be elevated while the pyrogen was present in the joint to elicit a febrile response.

DISCUSSION

It is almost axiomatic that fever tends to be highest with infection in tissues that are unable to expand and have no drainage outlet. This is seen in infection of joints, lung abscess, obstructive infection of the urinary tract, dental root abscess and obstructive sinusitis. The reasons why fever should be high and persist until drainage is established in closed infections are poorly understood. It is possible to attribute this to accumulation of bacterial toxins, accumulation of products of tissue injury, or facilitation of diffusion of pyrogen into the lymphatics or circulation

in the absence of adequate drainage. A factor of probable importance, however, is that if a closed abscess or infection is under increased pressure, the pressure may in some way increase the transfer of pyrogenic substances into the circulation, and the relief of the pressure may reduce this transfer of pyrogen, thereby resulting in defervescence. That pressure may play such a role in the production of fever in infection is suggested by the rapid fall of temperature seen after aspiration of a closed abscess prior to control of the infectious process itself.

The studies reported in this paper illustrate the importance of intraarticular pressure in elicitation of fever with bacterial pyrogen injected into the joint. That this is an artificial experimental model is admitted, but the findings clearly support the concept that the pressure inside a closed abscess may indeed be an important factor in production of fever.

Fever can be readily induced by inoculation of bacterial pyrogens (endotoxins) intravenously, and the mechanisms involved in this pyrogenic effect have been extensively investigated (3). That bacterial endotoxins can act upon leukocytes to cause the production of a pyrogenic substance is clear (5, 7) and the toxins may also act directly upon the central nervous system to elicit fever (8). The effect of leukocytic pyrogen and bacterial pyrogen is very likely conditioned, however, by the properties of tissues in which they are inoculated or produced. This influence is seen by the fact that bacterial pyrogen injected by another route than intravenously or intracerebrally may not elicit a febrile response (2, 3). Although this effect of route of inoculation could be attributed to the rapidity with which the pyrogen enters the circulation, it is also likely that changes in the properties of tissues may significantly influence the ability of pyrogens to produce fever.

Endotoxins are lipo-polysaccharide-protein complexes ordinarily of relatively high molecular weight, and leukocytic or endogenous pyrogen is a non-dialyzable substance, probably protein in nature (3, 7). It is difficult to understand, therefore, how increase in intraarticular pressure alone could enable the diffusion of pyrogen through the synovial membrane of the joint. The possibility that pressure enables the induction of fever, under the circumstances of the experiments described here, by injuring the synovia seems unlikely, however, as it was found that increase in joint pressure for a period of time, then allowing its return to normal, was not accompanied by development of fever after injection of bacterial pyrogen. It is true, nevertheless, that endotoxin has been shown to produce inflammation in a joint (9), but this alone cannot account for the induction of fever as endotoxin in the joint without increase in intraarticular pressure was unassociated with febrile response of the animal.

That pressure is responsible for increased diffusion of certain substances such as glucose and probably electrolytes out of a joint has been suggested before (1). Glucose and electrolytes, however, are dialyzable and of small molecular size. Therefore, it remains difficult to account completely for the effects of pressure on the febrile response observed in the studies reported here. It is probable that substances of large molecular size diffuse out of tissues into the lymphatics and not directly into the vascular system and there is ample evidence that in the presence of inflammation and increased tissue pressure lymphatic flow is accelerated (10). It is possible, therefore, that the escape of pyrogen from a joint under pressure is through the lymphatics, but this suggestion requires experimental demonstration.

Whether or not leukocytic or endogenous pyrogen could produce fever after injection into a joint without increasing intraarticular pressure is unanswerable at this time. Efforts to evaluate this problem have been unsuccessful as it has not been possible to prepare or obtain leukocytic pyrogen in sufficient concentration in a small volume. Subsequent studies, however, will be done to clarify this issue, but it seems unlikely that endogenous pyrogen will be less susceptible to the influence of tissue pressure in its ability to induce fever.

Whether or not there is any correlation between intraarticular pressure and fever in joint infection is not known. Intraarticular pressure is known to be increased in human pyoarthritides (1). Preliminary studies in our laboratory on staphylococcus pyoarthritis in rabbits have also suggested such a correlation in that development and height of fever seem to bear a close relationship to intraarticular pressure. Such experiments, however, are fraught with difficulties in that infection can extend beyond the joint, even to the development of systemic disease, and this complicates any interpretation of the febrile response. Efforts should be made to evaluate the pressure-fever relationships in human joint disease.

Intraarticular pressure in joint infection undoubtedly has other important effects than its probable implication in production of fever. For example, it has been shown that pressure can play a significant role in joint destruction during the course of infection (11). It would be well if further attention were paid to the physical phenomena of inflammation to elucidate their influence upon the evolution of disease.

SUMMARY

Increase in the knee joint pressure in rabbits by the intraarticular injection of normal serum does not elicit a febrile response in the animal. Similarly, intraarticular injection of bacterial pyrogen into the knee joint of rabbits does not induce fever under the conditions of these experiments. When the injection of pyrogen into the joint is accompanied by an in-

crease in joint pressure there is significant elevation in the rabbit's temperature. The implications of joint pressure in production of fever attributable to intraarticular pyrogen are clear, and the possible relationship of these observations to an understanding of the pathogenesis of fever in joint disease is discussed.

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CONGENITAL ARTERIOVENOUS FISTULA IN THE NECK—48 YEAR FOLLOW-UP OF A PATIENT OPERATED UPON BY DR. HALSTED IN 1911

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In 1918 at the autumn meeting of the National Academy of Sciences at The Johns Hopkins University, Dr. Halsted, one of the few surgeons who have had the distinction of Fellowship in this Society, presented a paper entitled "Congenital Arteriovenous and Lymphaticovenous Fistulae,—Unique Clinical and Experimental Observations". This paper consisted of a scholarly discussion of enlargement of the heart in cases of arteriovenous fistula and persistent ductus arteriosus, explanation of the presence of blood in lymph cysts at the time of aspiration, with notes and drawings by Dr. Florence L. Sabin, and comments upon the proximal arterial dilatation associated with arteriovenous fistula.

In connection with this latter subject he presented in some detail the instance of a girl upon whom in 1911, when she was 11 years old, he had operated, for a congenital arteriovenous fistula below the angle of the jaw on the right side. The fistula had recurred and had been reoperated upon in May of 1918 by Dr. Mont Reid. The paper is illustrated by two of Max Brödel's beautiful drawings (Fig. 3C, Fig. 4C). After 1922 the patient was apparently lost from observation for a period of 30 years. She has now been seen at intervals in the last 8 years with a large recurrence or persistence of the arteriovenous fistula. The beginning of bleeding from the external auditory canal recently prompted her to accede to the recommendations for an operation which she had been rejecting.

The original history and its contained photographs, which happily escaped destruction at the time the old hospital records were microfilmed, was located in the file of old histories at the Welch Library.

A few days after the birth of the baby on February 27, 1900 at a home delivery by a midwife, the mother noticed a throbbing on the right side of the baby's neck. There was a palpable buzzing in this area. In the 3 years before the 1911 admission to the hospital a swelling had been particularly prominent. The pulsation was noted to increase greatly during attacks of croup or earache. She had had three attacks characterized by dizziness which caused her to fall to the floor without loss of consciousness. The giddiness would last 5 to 10 minutes and the whirring and throbbing in

FIG. 1. Photograph in the history (1911) of the child at age 11. There was a strongly pulsating mass on the right side of the neck, just below the ear and behind the angle of the jaw, measuring three and a half by four and a half centimeters. There was no filling of the superficial veins to any abnormal degree. A loud continuous bruit and a strong thrill were present over the swelling.

By pressure upon the carotid below the tumor all pulsation in the aneurysm could be abolished.

the neck had become more pronounced at these times, while the swelling appeared as if it were about to break. She had occasional attacks of ringing in the ears. The physical examination was negative but for the lesion in the neck. Visual fields were carefully charted and were normal. Dr. Baetjer's x-ray report of November 4, 1911 states, "Carotid aneurysm present, but not shown in x-ray". There are rather satisfactory photographs much yellowed with age, but still showing the swelling beneath the angle of the jaw and the attempt of the examiner to compress the carotid (Fig. 1). The Special Note of November 3, 1911 by Dr. George Heuer stated,

"Patient complains of earache, headache, toothache, and pain in right eye. Patient has quite frequent earaches associated with which at times there are giddy spells during which she falls to the floor. Never been loss of consciousness but there is unsteadiness with the attacks."

"Objective: On the right side of the neck just below the lobe of the ear is a strongly pulsating mass which measures $3\frac{1}{2} \times 4\frac{1}{2}$ in transverse and vertical diameters. This mass pulsates synchronously with the heart beat and indeed is a very forcible pulsation pushing out the lobe of the ear with each throb.

"On inspection of the neck there is perhaps slight fullness of the right external as compared with the left. There is throbbing of both carotids, distinctly more marked

on the right than on the left. There is on inspection of the face, forehead and temporal region nothing especially remarkable. There is no dilatation of the superficial veins on either side. There is no filling of the veins on the right side of the face or temple as compared to the left. There is no difference in color of the two sides of the face. The pupils are equal . . .

"On palpation the tumor is definitely pulsating with an expansile pulsation. The most marked pulsation is present just behind the lobule of the ear and on placing the finger over this point a distinct whirring thrill can be felt. This thrill is confined to a rather small area, not more than 1½ or 2½ cm. in diameter. A definite aneurismal sac is felt and it lies directly behind the ascending ramus of the jaw. On attempting to trace the affected vessels upwards no very definite information is obtained, however, by pressure forward towards the angle of the jaw, immediately over the aneurismal sac, thrill can be absolutely abolished although the pulsation due presumably to the carotid continues. By pressure upon the carotid below the tumor all pulsation in the aneurysm can be abolished. On pressure over the external jugular below the tumor at the base of the neck, external jugular fills out enormously, markedly more than on the other side. When the vessel fills in this manner there is a definite pulsation of the vessel, although it is extremely difficult to determine whether this is a bruit (sic) of the vein itself or a transmitted one from the artery in the neck. An attempt was made to determine this point by pushing the carotid inward so as to rule out any transmitted pulsation but strong enough pressure to rule this out stopped the pulsation of the aneurysm.

"By palpation over the dilated vein no definite bruit could be made out. Above the aneurism no definite vessel can be palpated, the aneurismal sac itself reaching the highest point between the mastoid and the ascending ramus of the jaw. With the finger in the external meatus of the ear although no pulsation can be made out the temporal artery on the right is fully as strong as that on the left.

"Auscultation over the mass shows a remarkably loud rough continuous bruit definitely accentuated at the time of systole."

Operation was performed on November 15, 1911 by Dr. Halsted. The operation lasted 2 hours. The anesthesia was gas and ether. Dr. Heuer was the first assistant, and in accordance with the custom of the day, the first assistant wrote the operative note. A curved transverse incision, made across the neck below the jaw, passed upwards to the tip of the mastoid process. The external jugular vein was ligated and divided. Two sentences of the account are noteworthy as showing Dr. Halsted's care in regard to hemostasis.

"It might be stated here that throughout the dissection, which was done with extreme care, all the vessels encountered were ligated with double ligatures, the vessel wall itself being transfixed so as to preclude any possibility of the slipping of the ligatures. Due to this careful procedure at no time was there any hemorrhage whatever during any stage of the dissection."

When the skin flaps were reflected there was found a plexus of enormously dilated veins which was dissected free. One large vein passed from this plexus to the internal jugular which was noted to be surprisingly small. "The operator's plan of attack was to isolate the common carotid." (Fig. 3A, B, C)

"On carrying the dissection further this vein definitely joined the external carotid at a point directly under the posterior belly of the digastric muscle. This point was a short distance above where the hypoglossal nerve crossed the vessel and above the point where the lingual artery came off the external carotid, and rather hidden by the jaw . . . There was demonstrated, therefore, a definite communication between the carotid artery and this sinus-like vein the attachment being by quite a broad area. A double ligature with transfixion of the vein was done about 1 cm. or 1½ cm. below the point of communication of artery and vein and the vein below this was excised. There remained, therefore, only a stump of vein which communicated with the artery. The external carotid itself was then ligated just below the hypoglossal nerve with a single ligature of heavy black silk, the ligature not being drawn too tight. When this was done, all pulsation and thrill, and all tumor in the neck entirely disappeared. The external carotid above the aneurismal varix was not seen or ligated."

The first two postoperative progress notes are in Dr. Heuer's hand and thereafter the intern, Dr. J. C. Colston kept the chart. Heuer notes on the first dressing on November 21

"With removal of dressing it was found that a tumor was still present though smaller than before. Slight visible pulsation persists—not at all the violent pulse previously noted. On palpation Dr. Halsted felt a very fine almost imperceptible thrill—not felt at this time by Heuer. On auscultation a distinct bruit but very much less loud than before. The diastolic part of bruit is very much less."

In his discharge note, December 12 Dr. Colston remarked

"The rt. side of the neck is still slightly fuller than the left and there is still rather marked visible pulsation. On palpation a definite thrill can still be felt and tumor, though smaller, is still definitely expansile. On auscultation bruit is still present. Discharged."

In the out-patient department, April 26, 1915 it was noted that she had been entirely relieved of tinnitus and pain in the right side of the face and the neck was smaller than before operation. However, there was occasional swelling of the right side of the face which was not present previously. On this occasion the right side of the face and neck felt somewhat boggy to the examiner. The great enlargement of the carotid artery on the right was palpable and was thought to measure 2½ cm. as opposed to 0.8 cm. on the left.

In response to repeated suggestions of reoperation her mother finally brought her to the hospital again in 1918 (Fig. 2A, B, C). The patient then stated that she had been free of pain and earaches since the operation but that a short time after operation her right face had become swollen and that for the past few years her right eyelid had been very much swollen. At times the superficial veins of the face were very prominent. She was in hopes that operation would reduce this swelling of her face. Dr. Mont Reid had seen her in the out-patient department and left a two-page typewritten note.

A B

C

FIG. 2. The patient's condition in 1918—photographs in history. A. Full face. The written legend states: "Front view of the aneurysm. Note the hypertrophy of the right side of the face, the drooping eyelid due to the telangiectatic condition. The aneurysm shows well. The dilated temporal vessels can be seen." B. The legend here reads: "With the fingers compressing the carotid artery the aneurysm goes down, the dilated veins in the temporal region collapse, and the cyanosis of the right side of the face is much less. The patient feels better except that a 'scum' seems to come over her eyes." C. Side view. The legend in the chart reads: "A = Apparently the situation of the fistulous opening, as determined by pressure." "B = Area of pulsation." "C = Dilated carotid artery."

He noted that for the past few weeks she had a little headache almost every day, that she heard no noise, but when she placed her hand on the swelling she could feel "rushing." He noted "the difference in appearance of the two sides of the neck and face is striking. The whole right side of the head including the ear is suffused and slightly cyanotic with numerous dilated skin venules suggesting telangiectasis. About the outer canthus of the right eye, involving both lids, coils of veins can be seen, so that the condition resembles somewhat hemangioma. There is a generalized slight edema of the entire right face. Dilated veins can be seen extending up into the hair and up as far as the midline. Under these large veins, which run up over the skull, a definite groove can be felt in the bone. The aneurysm can be seen pulsating readily, and the pulsation of the right common carotid is quite striking while that of the left is barely visible. The whole right ear moves with each pulsation. Slight pulsation can be seen in the veins in the front of the ear.

"Dr. Halsted thinks the aneurysm is getting decidedly larger, and this seems quite striking to him since he saw her the last time, about a year ago. The aneurysm now occupies almost exactly the region of the right parotid. The point of maximal swelling now seems to be higher than the photographs showing it when she was operated upon 7 years ago, and it seems to Dr. Halsted that the swelling has wandered up so to speak. The most prominent point now is right at the lobe of the ear. As compared with the photographs in the history the swelling is decidedly larger, and the dilatation of the veins in the face is also markedly more pronounced.

"The carotid artery on the right seems now to be about 2½ to three times larger than the one on the left. When it is compressed the aneurysm quickly goes down and ceases to pulsate. During this procedure, the patient complains of a dark shadow coming across the right eye. It is interesting to note that when the carotid is compressed the aneurysm subsides, distended veins collapse and cyanosis of the patient's face and ear largely disappears; returning quickly when compression is released. With the artery compressed no thrill can be felt, and no pulse in the aneurysm, but a further faint bruit can be heard just below the lobe of the ear in an area about as large as the bell of a stethoscope. When the artery is compressed the patient says that the right side of her head and face feel 'lighter'. Immediately when the compression is released she rotates her head a little and complains that the side of her head feels 'heavy'. Over the point of swelling is a marked continuous arteriovenous hum with systolic intensification. This can be heard over the temporal artery and its branches well above the ear. It can be heard along the course of the carotid down almost to the clavicle.

"It is very easy to demonstrate the point of fistula by pressing just below the lobe of the ear just behind the angle of the mandible. When the small bell of the stethoscope is pushed in at this point firmly the fistula is closed and no sound except the heart beat can be heard."

Dr. Arthur Bloomfield (then resident in medicine) was the medical consultant. He stated that there was no evidence of any cardiac abnormality.

Fundoscopic examination by the ophthalmologist Dr. Reed, showed that "the veins in the right eye are huge. When the carotid artery is compressed these veins collapse to almost normal size, filling quickly on releasing the pressure. The cup seems a little swollen but the margins of the disc are clear. The left eye is normal."

The electrocardiogram showed no abnormalities. The modern looking phonogram taken over the aneurysm shows almost constant murmur dying off in early systole but

very intense in late systole and early diastole. Compression over carotid artery caused all trace of the murmur to disappear."

On this occasion Dr. Ella Oppenheimer was the clinical clerk. Dr. Reid performed the operation on May 24, 1918.

"An inverted question mark incision was made with the straight portion extending up by the ear in front and curved part extending around the lobe of the ear toward the mastoid in the middle of the neck. The angiomatous condition of the skin made oozing at every place the incision was made. The superficial skin flap was reflected forward over the parotid gland and the aneurysm, avoiding the facial nerve. The huge common carotid artery was freed and a tape passed about it. The dissection was carried along the vessel and posteriorly to the mastoid process while the facial nerve was seen and exposed. The digastric muscle was encountered and divided. The hypoglossal nerve was exposed and found to be densely adherent to the carotid so that dissection was difficult. "About 2 centimeters down the neck from the point where the hypoglossal nerve crossed the artery, the internal carotid artery was given off and the dissection of it was not so difficult; in fact the tissues about it would have led one to think that this vessel probably was not exposed in the former operation."

"About the level of the hypoglossal nerve, the now external carotid artery suddenly diminished in size in a beautifully cone-shaped fashion from a huge artery to ½ to ¼ its size. Just at the point of this diminution in size, there was considerable scar tissue, and it is likely that in dissecting out the vessel some portion of this scar tissue represented the old original external carotid artery which was ligated. At no time did I see any parts of the original silk sutures. The small artery which was then probably an aberrant vessel, and which coursed in the position of the external carotid vessel, ran directly up into huge venous lakes beneath the parotid gland and between the angle of the jaw and mastoid process. One of these large venous trunks—perhaps as large as one's thumb—extended down and medial to this small artery. Beneath the parotid gland these huge venous lakes were dissected out as well as I could and the small artery was traced directly up by the side of one of them. In order to prove the relation of this small artery to the arteriovenous fistula, we occluded it and noticed that the thrill which was easily felt in the dilated veins disappeared. We also occluded the internal carotid artery and this resulted in a definite increase in the intensity of the thrill. There could be no question but that the small aberrant artery connected directly with the veins. This small artery was doubly ligated and divided. Also the large venous trunk running medially to it was similarly treated. With the distal end of these two vessels as retractors, I was able to pull down the large venous dilatation and the artery from beneath the parotid gland and the facial nerve. I ligated several of these huge venous trunks which went into the dilated venous pocket, and also ligated the artery well up under the parotid gland thus removing in one piece the artery and a large venous sac.

"Dr. Halsted who was watching the operation was inclined to think that the fistula was between the two vessels that I removed; of this I was not so certain. I, however, was certain that ligating the artery and vein proximal to the fistula, together with the excision of a large portion of the aneurysmal veins, would certainly cure the patient. After this procedure there was no evidence of any thrill to be felt at any point . . .

"In this case the common carotid artery was enormously dilated. The internal carotid artery was slightly dilated. The external carotid up to the origin of the aberrant vessels was almost as large as the common carotid artery. The aberrant vessel, as I so

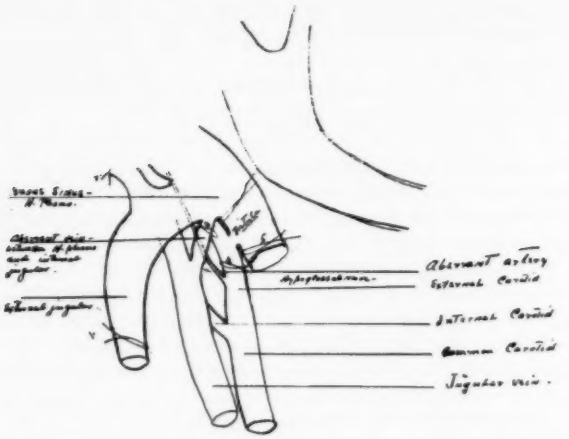
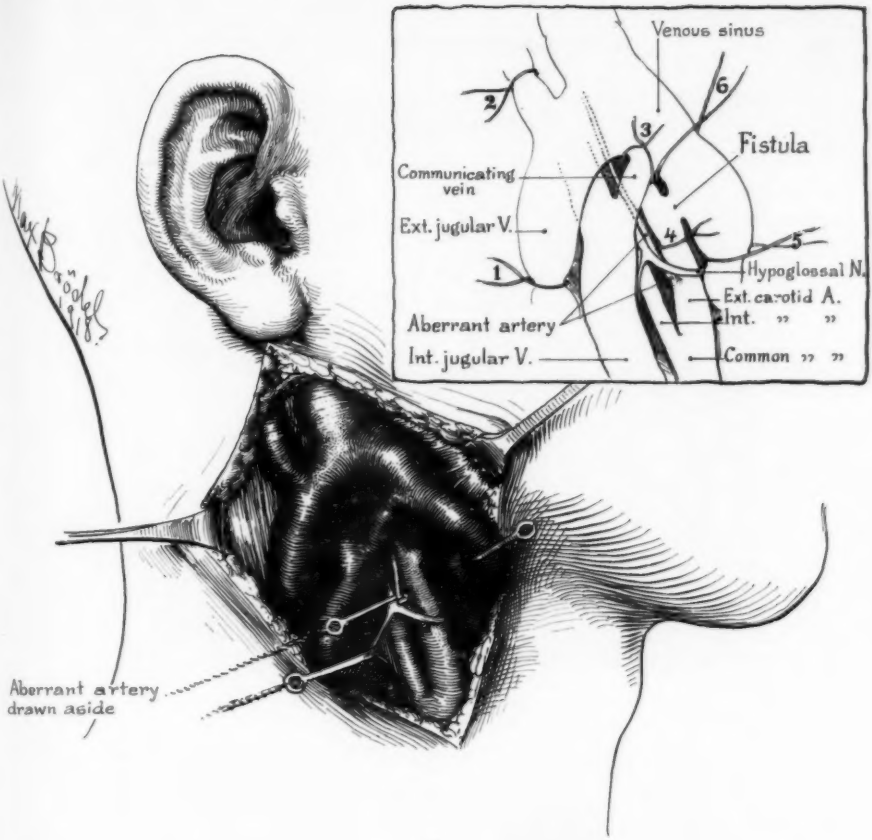

Aberrant artery
External Carotid
Internal Carotid
Common Carotid
Jugular vein

FIG. 3A

Op. Nov. 3. 1911
Fistula
External jugular V.
Internal jugular Vein

FIG. 3B



C

FIG. 3. The first operation (Dr. W. S. Halsted). A. Diagram by Dr. Reid of the condition in 1911. A portion of the original sheet has been torn away, but reference to Figure 3B & C will show the fidelity with which Mr. Brödel reproduced the diagram in the small insert, and then made an artistic rendering. The handwritten notes on the sheet containing the drawings state: "Operation November 3rd, 1911 (drawing made by Reid on May 28th, 1918 and based on the operative note made by Dr. Heuer). "1, 2, 3, 4 and 5—probable points of ligation in the first operation. The connection between the aberrant artery and the venous sinus was higher in the neck, and thus it explained the shifting of the swelling upward with the development of the second aneurysm. Dr. Halsted's fistula was lower, and was undoubtedly cured. B. Max Brödel's sketch, in the 1918 record, of the first (1911—Dr. Halsted) operation, after the sketch made by Mont Reid (3A). This pencilled sketch, the vessels crayoned red and blue is on a sheet of paper, loose in the clinical record. C. Brödel's finished drawing as it appears in Halsted's 1918 publication. The relationship of the small insert to Reid's sketch is obvious. This drawing (and 4C) are reproduced from Mr. Brödel's originals in the possession of the Department of Art as Applied to Medicine, and were kindly made available through the courtesy of the Director, Mrs. Ranice Birch Davis.

The original legend read: "Congenital fistula between the external carotid artery and a large vein. Appearances at the first operation. Dilatation of the common and external carotid arteries central to the fistula, and of the venous plexus. The internal carotid is abnormally small. There is a tiny aberrant branch of the external carotid."

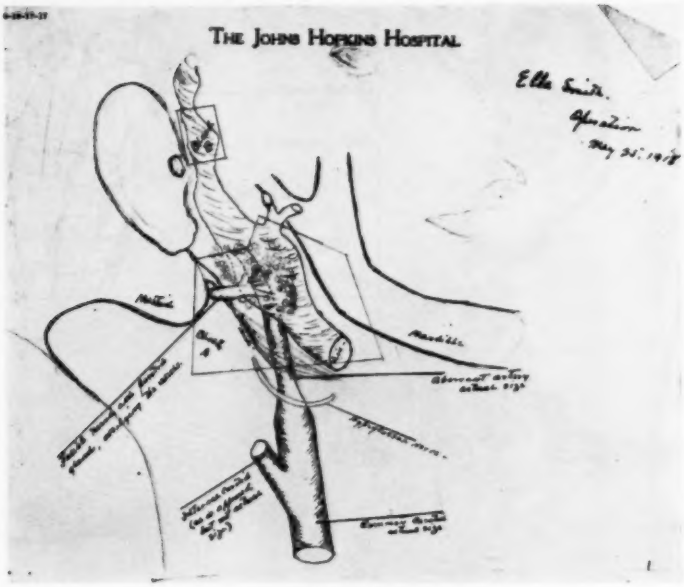
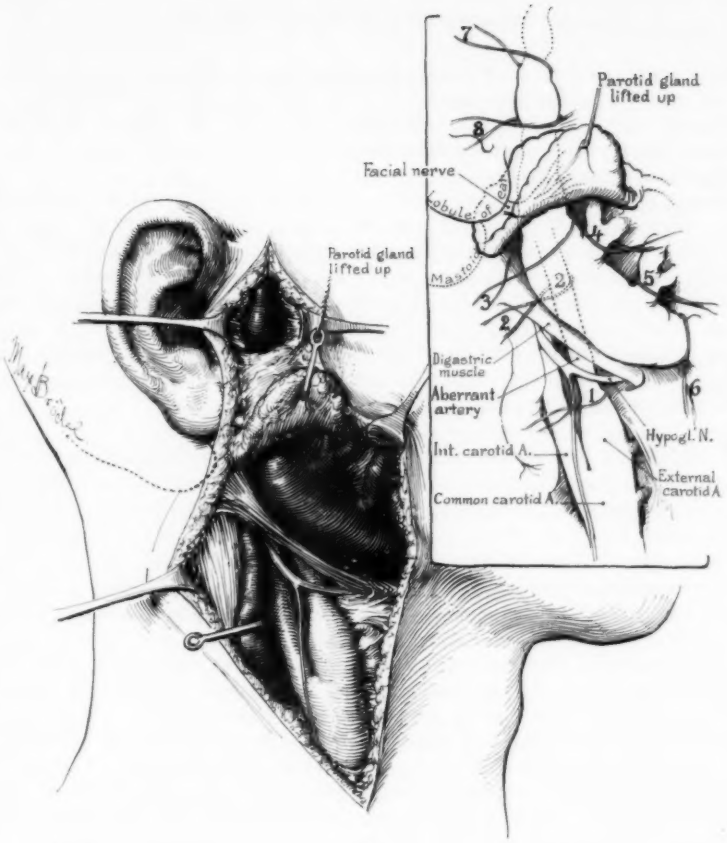


FIG. 4A

FIG. 4B



C

FIG. 4. The second operation (Dr. Mont Reid). A. Dr. Reid's drawing of the operation May 25, 1918. The carotid is shaded in red ink. The drawing is on white unlined history paper. The handwritten legend reads: "All of the carotid arteries were exposed and the vessels in blocks A and B, but between blocks A and B the vessels were not exposed. It may be that the actual connection between the arterial and venous systems is at some point between blocks A and B. The vessels in blocks A and B were removed. The external carotid artery was not seen and the only remains of it were probably scar tissue at the level of the sudden decrease in size. The large aberrant artery seemed to be a direct continuation of the external carotid. The development in the size of this vessel since the first operation is remarkable. The sudden decrease in the size of the artery is to be explained by the ligation of the external carotid at the level of the hypoglossal nerve during the first operation." B. Brödel's sketch probably made from Reid's drawing (4A) but possibly drawn in the operating room. It is on white sketching paper pasted on a sheet of lined blue surgical history paper. C. Brödel's finished drawing as it appears in Dr. Halsted's 1918 publication (reproduced from original drawing, see 3C). Once more the parotid was only reflected and the area behind it not dissected. The original legend stated, "Appearances at the second operation. The internal carotid and the aberrant branch of the external carotid have become markedly enlarged, the fistula having shifted its position to a peripheral point."

called it, was about the size of a normal external carotid artery in a girl this patient's age and size. The sudden diminution from a big artery to a small vessel was most striking (see the diagram made from actual measurements) (Fig. 4A, B, C). It is interesting to note in the previous operation the external carotid was huge and that the internal carotid was extremely small.

"As I think about this case it occurs to me that it would be rather remarkable to find a congenital side by side anastomosis between vein and artery. What seems more likely to me is that this aberrant artery runs directly into the venous system. At this point I think that there was definitely established the huge arteriovenous anastomosis or direct communication on the order of end-to-end anastomosis rather than side-by-side fistula."

The pathologic study of the histologic section "shows nothing remarkable, except thick walled veins, definite hypertrophy of the few muscle fibers, and increase of elastic tissue of the walls of the vein."

Dr. Reid has a note that the relationship of the vessels had not been preserved and therefore there was no section at the point of communication between the artery and the vein.

Six days after operation Dr. Reid records in the first postoperative note "the dilated veins of the face are collapsed and the skin is 'white'. No pulsation and no thrill can be felt in the region of the original swelling. A very faint bruit however can be heard." On the 8th of June in his next note Dr. Reid states that "on inspection and palpation the aneurysm seems to be cured. Yet a definite bruit can be heard. Symptomatically she is well. The eye is clear and the right side of the face does not feel heavy. Compression of the carotid artery lessens the bruit but does not make it disappear." On May 30 Dr. Henry Hun, the intern, recorded that "since operation the patient's tongue has been much swollen and this has caused her much discomfort. It is ulcerated at the base. The congestion on the right side of the face has largely disappeared. There is still a distinct bruit audible at the angle of the right jaw but this is very much less in intensity than before operation".

On June 13 Dr. Reid was able to feel a little thrill just in front of the lobe of the ear "corresponding to the segment of vein which was not removed at the time of operation. A definite bruit is here present. The vein at this point is definitely felt—no larger than the distal segment of one's little finger. There, however, is certainly definite connection between the arterial and venous system."

From the history we have reproduced the diagrammatic drawings of this operation and of Dr. Halsted's operation both apparently made by Dr. Reid on the occasion of the second operation (Fig. 3A, Fig. 4A). His drawings of the earlier operation are stated to be made on the basis of Dr. Heuer's notes. They are then included in the patient's chart with beautiful pencil sketches by Mr. Brödel (Fig. 3B, Fig. 4B) of the two operations, in which the vessels are colored with red and blue crayon. These sketches done by Mr. Brödel, both probably from Reid's diagrams, were obviously the working sketches for the finished drawing (Fig. 3C, Fig. 4C) illustrating Dr. Halsted's 1918 report.

Dr. Reid saw the patient in October, 1920. "One is struck by the marked difference in pulsation of the 2 carotid vessels:—the right pulsation very perceptibly more than the left. It seems as if one can actually see that this is actually larger. The difference in the color of the two sides of the face persists, this difference being especially marked in the right eyelids which are more bluish or angiomatous in appearance than the left. There is also a little puffiness of the lids. The paralysis of the infundibular branch of the right side is now scarcely perceptible." This partial facial palsy was not previously recorded in the chart nor was the hypoglossal palsy mentioned as such.

"A slight swelling just below the lobe of the ear . . . seems possibly to have increased a little bit since I last saw her. The swelling pulsates slightly but not markedly. A beautiful thrill is easily felt, continuous in character. A loud continued bruit is heard over this swelling and with diminished intensity for a distance of about 1½ inches on all sides of it. The carotid artery is definitely enlarged, appearing to be about 2½ times the size of the left one. Occlusion of the common carotid on the right causes some blanching of the face but does not make her feel unusual. When this occlusion is released the change in her expression is noticeable and she says it feels as if something is rushing through the face".

Dr. Reid saw her again on March 14, 1922 (Figs. 5A & B). She had only occasional small headaches and suffered no longer from dizzy spells. She no longer had the queer sensation in her head when she bent over, that she used to have. "The condition impresses me as having made some advance since the last note. This is shown particularly in the angiomatous condition on the right side of her face, the upper eyelid and extending slightly across the midline of the forehead. In the upper eyelid and at the outer end of the eyebrow the condition is a typical angiomatous one. No pulsation, however, can be seen in these vessels. The point of fistula—that is just below the lobe of the ear—seems to have increased in size. As compared to the last observation I should say that swelling has increased about a third in size. Very definite thrill is palpable. A loud typical arteriovenous bruit can be heard. This bruit is transmitted upward and can be heard over the right temporal region. It is not well transmitted proximally. It cannot be heard over the middle of the right common carotid. I get the impression that the upward conduction is a bony conduction."

"The difference in the size of the 2 carotid vessels is very striking. On inspection it is easy to see that the right carotid artery is much larger and beating with tremendous force as compared with the left. On palpation this vessel appears to be about three times as large as the left carotid. The left carotid is about the normal size . . . Occlusion of the right carotid artery diminishes only slightly the audible bruit. Occlusion of the left carotid diminishes the bruit almost to the same extent. Occlusion of both carotids makes the bruit disappear. From these observations I should feel that there is a very free communication between the blood supply of the two sides of the face, and that the source of blood coming into the fistula is almost as good from the left side as from the right side.

"The patient's heart seems to be perfectly normal.

"The angiomatous condition of the face makes me feel that in this case we may possibly be dealing with a pulsating naevus instead of a single arteriovenous fistula. Yet the point of maximal bruit and thrill is very definite and seems to be directly over a very large communication between the arterial and venous systems."

She was not seen at the hospital again until November 21, 1951 when she was referred to one of us by her family physician. She was then 51

FIG. 5A

FIG. 5B

years of age and stated that she was in good health and had never been short of breath and in fact had no cardiorespiratory symptoms. She thought the mass had not particularly enlarged in size since the last operation, but was coming in for advice because of a number of attacks of sudden, intense awareness of a constant bruit in the region of the mass with a great increase in rapidity in the heart beat. These attacks usually came on in association with emotional tension.

There was a visible swelling in front of and below the right ear extending up as far as the outer canthus of the right eye. There were numerous visible veins over it but these were not tremendously enlarged or engorged. There were 2 transverse scars in the right side of the neck and another scar around the lower portion of the ear and up along the anterior portion of the ear of the tragus. The mass in the region of the right carotid at the angle of the jaw was soft and compressible and over it was a readily palpable thrill and a continuous bruit. The thrill and bruit were maximal behind the ear and behind the angle of the jaw. Pressure deep in the neck at about the level of the angle of the jaw or below, on the palpable carotid artery, diminished the bruit very sharply but did not eliminate it altogether. The note states that "Dr. Blalock examined the patient also. I rather think that he felt he was capable of completely obliterating the bruit but I was not able to do this." There was still evidence of the old facial palsy in the lower lip at the corner of the mouth. The patient stated that this had been worse after the last operation and that for a long time after the last operation she had had great difficulty in using her tongue which when protruded had projected to the right side. Her ear drums were quite innocent and her external canal only very slightly narrowed. Her fundi were both normal. She was admitted to the hospital for study.

X-rays showed no bony abnormalities. The right cerebral arteriogram showed a considerably enlarged right common carotid artery. The stump of the external carotid artery appeared to be visualized. The internal carotid artery pursued the normal course. The intracranial portion of the in-

FIG. 5 A and B. Photographs taken March 14, 1922. Doctor Reid stated at this visit "The condition impresses me as having made some advance since the last note. This is shown particularly in the angiomatous condition on the right side of her face, the upper eyelid, and extending slightly across the midline of the forehead . . . As compared to the last observation [in 1920] I should say the swelling has increased about a third in size. Very definite thrill is palpable. A loud typical arteriovenous bruit can be heard . . . On inspection it is easy to see that the right carotid artery is much larger and beating with tremendous force, as compared with the left. On palpation this vessel appears to be about three times as large as the left carotid . . . Occlusion of the right carotid artery diminishes only slightly the audible bruit."

ternal carotid artery seemed quite normal. There was prompt filling of anomalous vessels in the region of the distribution of the external carotid artery. Some of these vessels were quite large and were thought to be greatly dilated veins. "Exact method by which these fill is not clearly demonstrated but I suspect that it is by means of collateral circulation since the main trunk of the external carotid artery is not seen." (Dr. Robert Sloan)

The patient was advised that the lesion was potentially dangerous and that operation should be undertaken. In view of her general freedom from any severe symptoms over so long a period of observation this recommendation was not pressed and although the patient returned for examination from time to time she never found it convenient to arrange for operation.

On October 13, 1959 she called up for examination because of bleeding from the external ear. Examination showed a few drops of blood issuing from a crusted area on the anterior wall of the external auditory canal. This was in an area of some narrowing of the canal. The examination of

FIG. 6A

her face showed very little change to have occurred over the past eight years (Fig. 6, A & B). The mass was probably somewhat larger and more diffuse. The entire parotid region was a tense, pulsating, compressible bag of blood under arterial pressure. There was no extensive engorgement of superficial vessels. The eye grounds showed no abnormalities. A thrill could be felt quite easily in the region of the angle of the mandible and back under the ear lobe toward the mastoid. In the same area there was audible a remarkably loud continuous machinery murmur. The pulsation

B

FIG. 6 A and B. Admission photographs, October, 1959. A slight facial palsy, residuum of the 1918 operation, was scarcely perceptible. The aneurysmal swelling had greatly increased in size and extent. The thrill and bruit were intense, and maximal, as before, between the mastoid process and the angle of the jaw. The common carotid was a great, forcefully pulsating structure. Pressure upon it (the external carotid had been ligated by Dr. Halsted) very largely obliterated thrill and bruit. At operation it was found that complete occlusion of common or internal carotid artery eliminated all pulsation and bruit in the tumor. She had been bleeding from a crusted area on the anterior wall of the external auditory canal.

of the common carotid in the neck was tremendously forceful and many times more conspicuous than that on the left. The right common carotid artery could be very easily palpated between the fingers and seemed to be almost 2 centimeters in diameter. On compression of the right common carotid artery between thumb and forefinger the pulsation disappeared and the bruit decreased very sharply although I could not eliminate it entirely. The patient was able to tolerate compression of the carotid for over a minute without any distress. Compression of the left common carotid against the vertebra produced no change in the bruit.

Radiologic investigation of the jaw and skull once more failed to show any involvement. It did not seem worthwhile to repeat the arteriogram since in general these studies fail to show the site of communication in arteriovenous fistulae.

It was felt that the beginning erosion into the external auditory canal represented a life threatening complication and that operation was mandatory. It was anticipated that there might be serious difficulty in hemostasis and that bilateral carotid occlusion might be required and for this reason the operation was done under hypothermia, temperature 32° to 33°C.

Operation was undertaken on October 16, 1959. It was planned to make a long oblique incision in the neck continued upward as a Y around the ear. This plan was followed with the addition of a transverse extension from the middle of the cervical incision across to the anterior midline. The only significant difficulty with hemostasis was in the skin incisions which bled furiously until the skin could be reflected and the innumerable vessels coagulated.

The common carotid artery was found to be enormous—fully 2 centimeters in diameter. At the bifurcation of the common carotid the external carotid continued of similar size for a distance of about 1 cm. when it abruptly tapered off and disappeared. There was no connection to any distal vessel, the ligation performed by Dr. Halsted having long ago completed the division of the vessel. It would appear from Dr. Reid's note that he divided the scar in his operation. The internal carotid proceeded up from the bifurcation as a slender vessel no larger than normal and perhaps a little smaller than normal. Two rather thin-walled, tortuous, almost varicose vessels arose from the bifurcation of the artery and passed upwards into the large plexus of the great thin-walled vessels containing pulsating blood just under the angle of the mandible. These two vessels were divided and proved to be in direct communication with the carotid. The internal jugular vein was not large, not tense, and did not pulsate.

Dissecting medially and upwards, looking for the distal trunk of the external carotid (which Dr. Reid also failed to find) we ran into a large extension of the principle aneurysmal varix which had tongues of large

thin-walled vessels extending down toward the mediastinum medially behind the esophagus and upward behind the pharynx. These communications were divided and ligated one by one as far medially as possible. It seemed reasonably certain that these were efferent vessels and that they need not be traced to their farthest attachments. As we came up toward the edge of the mandible we feared that we might find ourselves in a pocket and turned our attention therefore superiorly. In accordance with our previous plan, of which the patient had been informed, the facial flap was reflected anteriorly to well beyond the parotid gland and the pulsating swelling. Dissecting both with the cautery and with the knife the subcutaneous fat was cut down to the masseter muscle. We necessarily and

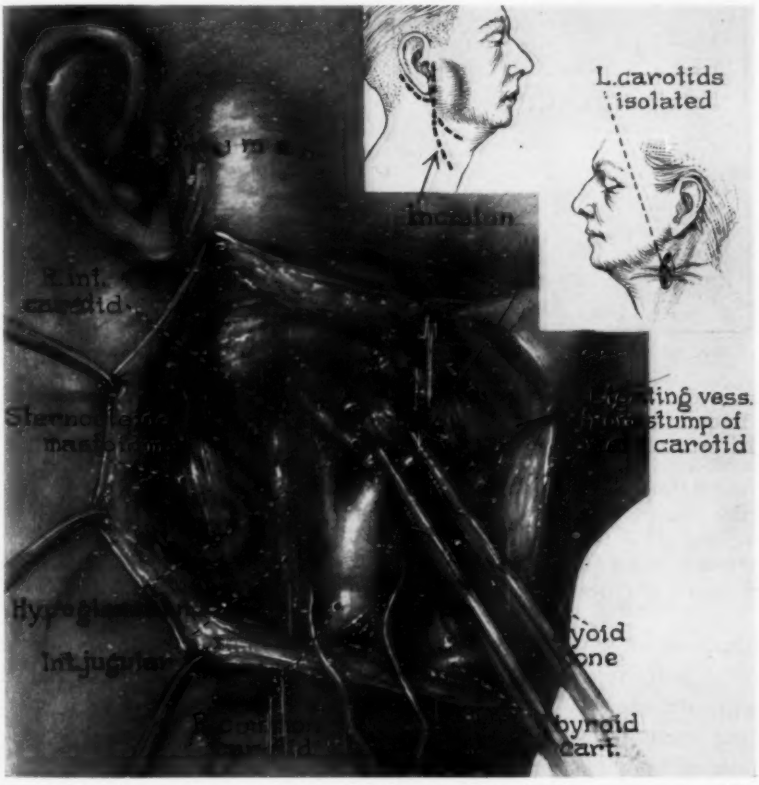


FIG. 7. Drawings made at operation, October 16, 1959. A. The common carotid was enormously dilated and the internal carotid, if anything, rather small. The external carotid had been ligated at the first operation and no trace of it could be found. Two small varicose pulsating vessels from the blind proximal end of the external carotid ran toward the aneurysmal mass. These were divided.

FIG. 7B. Numerous thin-walled vessels containing arterial blood under high pressure ran from the aneurysmal mass medially behind the pharynx. Note the neuroma of the hypoglossal nerve produced at the previous operation. The parotid and aneurysmal mass were reflected laterally to expose the vessels behind them. The deliberate sacrifice of the facial nerve seemed unavoidable.

deliberately divided the branches of the facial nerve. Underneath the parotid, as we reflected it backwards, and in the dense fascia between the zygoma and the mandible, we encountered three broad, obviously arteriovenous, communications. These seemed each to be flat multi-channeled bundles of vessels rather thick-walled but not obviously originally arterial. They were divided between clamps and transfixed and oversewn with silk. These channels seemed to be about a centimeter and a half wide but quite flat. Superiorly the parotid was dissected downwards, and posteriorly the skin flap was raised and the ear reflected so that one could come down along the anterior wall of the external auditory canal and the bony canal.

FIG. 7C. Between the zygoma and mandible lay two broad groups of thick-walled vessels. Behind the angle of the mandible ran three large thick-walled arteries. Compression of the internal carotid completely obliterated the pulsation in these arteries and in the mass and abolished the bruit when the mass was ausculted with a sterile stethoscope.

This dissection again threatened to lead us to a pocket and we now turned our attention toward the border of the mandible. To our surprise the finger could slip down below the edge of the mandible, anterior to the reflected parotid, between a great pulsating hollow mass and the border of the mandible. Dissecting carefully we now encountered an obvious artery a good centimeter in diameter containing blood under very great pressure. We had repeatedly demonstrated during the operation that with compression of the right common carotid artery the pulsation in the mass disappeared and the bruit listened to through a sterile stethoscope also

disappeared. We had at the very outset of the operation exposed and passed tapes around the left common and internal and external carotid arteries and found occlusion of these vessels did not affect the thrill or the pulsation or the bruit (Fig. 7, A & B). The large artery coursing from under the mandible, passing straight backwards parallel to the edge of the horizontal ramus of the mandible, was ligated, transfixed and divided. It appeared to be quite thick-walled. When this was divided a second such

FIG. 7D. The final operative field. Three substantial arteries entered the aneurysmal mass just anterior to the bony auditory canal. The hypoglossal, spinal accessory and vagus nerves were spared. The patient's clinical course indicated that the glossopharyngeal had been injured presumably in the dissection anterior to the bony auditory canal. No bruit was audible at any point after the aneurysmal mass had been removed.

artery came into view of only slightly smaller size and when that was similarly treated a third such vessel was seen and divided. We had, therefore, thus far found the two thin walled arteriovenous communications from the carotid bifurcation; the three broad rather flat channels coming through the fascia below the zygoma and now these three large discrete and unmistakable arteries. We now turned our attention posteriorly. The portion of the sternocleidomastoid muscle attached to the mastoid, which was involved in the posterior border of the pulsating sac, was divided. The spinal accessory nerve was spared. The entire specimen could now be elevated and was found to be attached only along the anterior wall of the bony canal of the ear. Here there were two or three very large rather thick walled vessels which might have been arterialized veins or might have been arteries. At all events these were divided and transfixed and the specimen removed. There was now no mass, no pulsation, no thrill, no bruit. It must have been in this last portion of the dissection that we injured the glossopharyngeal nerve. We had identified and spared the spinal accessory and the vagus. The hypoglossal nerve had been spared although the great venous trunks coursed above it and below it and around it and had to be untangled from it. Interestingly enough, in view of Dr. Reid's operative note and in view of the patient's difficulty with the tongue after the previous operation, there was a large and typical neuroma of the hypoglossal nerve.

There had not at any time been any significant loss of blood. The operation was a long and tedious one, taking 7½ hours. The patient had received 1 unit of blood prophylactically during the procedure and was given another at the conclusion. She was awake as the skin sutures were being placed, pulse and pressure were good, and she was readily rewarmed.

The postoperative convalescence was uneventful. A catheter placed under the skin flaps for suction drew almost no blood and healing was prompt. She had a complete facial palsy and a palatal palsy on the right. While the glossopharyngeal nerve was undoubtedly injured it was probably not divided and function has been partially restored. The only symptom seems to be a weakness in pharyngeal propulsion of solid food. By October 30, 1959 she was swallowing better, although not normally. The arterial pulsation in the right common carotid was still awesome. There was no thrill or bruit. Seen December 15, 1959 (Fig. 8, A & B) the bruit and thrill continued absent and the carotid pulsation has sharply decreased. The palatal palsy has cleared although there is still a little difficulty in swallowing solid foods. She is not troubled by the facial palsy and does not wish it corrected.

DISCUSSION

It is probable that there were originally multiple arteriovenous com-

munications, and from the previous operative notes it is quite plain that the operators recognized that these were never completely removed. The anatomical situation was such that these communications could not have been safely attacked without removal of the parotid gland and sacrifice of the facial nerve. At least it is not likely that the problem of hemostasis could have been very safely dealt with in any other way. Since she survived for 41 years after the second operation with symptoms and disfiguration less than that produced by a facial palsy, it may be that the decision not to extirpate the aneurysm then was the correct one. It is perhaps fortunate that when she did bleed externally the bleeding was trivial. It is, of course, possible that other abnormal communications still exist and will dilate in time. Since in the present procedure the entire aneurysmal mass was excised together with all attached vessels, more as if it were a malignant neoplasm than an arteriovenous fistula, it is thought quite probable that all communications have been removed. The decrease, for the first time, in size of the common carotid is further reassuring. The threat to life from hemorrhage has been removed.

FIG. 8A

Of greatest interest is the fact that the abnormal communications seemed to come from the internal carotid artery. It was repeatedly demonstrated before and during operation that compression of the internal carotid obliterated the thrill and bruit, and it was most striking after the specimen had been removed that the stumps of the three large discrete arteries which had entered the mass ceased pulsating each time the internal carotid artery on the right was occluded. Feeling under the floor of the mouth and behind and above the mandible one could feel the great arterial trunk running forward from the divided ends. Where these joined the internal carotid we did not demonstrate. Dissection of the internal carotid itself up towards the skull did not reveal the origin of these abnormal vessels or, as is more probable, of the one vessel with trifurcation.

B

FIG. 8 A and B. Postoperative pictures, December 15, 1959. The complete facial palsy resulted from the en bloc resection of the parotid and the vessels in and behind it. There is no swelling, no thrill and no bruit. The previously greatly enlarged and forcefully pulsating common carotid artery has sharply decreased in size and the vigor of its pulsation is no longer abnormal.

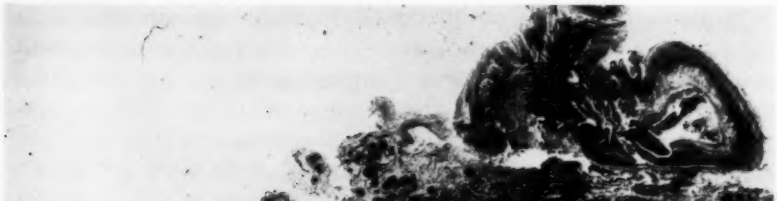


FIG. 9. Photomicrograph (×6) of portion of specimen. H and E stain. Note the great thick walled arteries, and the large veins, intimately attached to the parotid gland and to the facial nerve.

In view of the certain existence of abnormal branches of the internal carotid artery in the neck it is of more than passive interest that we could not find the trunk of the external carotid beyond Dr. Halsted's ligation. Neither could Dr. Reid, and Dr. Halsted himself remarked in the note which he dictated in the operating room "The external carotid above the aneurismal varix was not seen or ligated".

It is possible then that some external carotid branches arose abnormally from the internal carotid and that these abnormal branches were the ones which communicated with the varix.

NOTE, MAY 18, 1960

It is embarrassing to record that on February 5, 1960 the patient returned again with bleeding from the ear which this time was found to proceed from an extensive middle ear infection with destruction of the drum. The ear was deliberately not examined on the occasion of her previous admission. Roentgenograms now showed an extensive mastoiditis and on February 25, 1960 a radical mastoidectomy was performed by Dr. George Alderman. No significant bleeding was encountered. The patient remains well.

ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

April 11, 1960

THE COLLABORATIVE CEREBRAL PALSY STUDY

JANET HARDY

The Johns Hopkins Medical Institutions are among the group of some 15 institutions collaborating with the Institute of Neurologic Diseases and Blindness in an intensive study of 50,000 pregnant women and their offspring. The objective of this study is the illucidation of etiologic factors responsible for cerebral palsy, congenital malformation and other neurologic defects. If these factors and the mechanisms through which they work can be identified, sound preventive measures can perhaps be instituted to prevent these crippling and expensive conditions. Prevention is important when one realizes that 3 per cent of the 4.5 million children born each year in the USA, are mentally retarded and that the 168,000 cerebral palsied individuals cost approximately $126,000,000 per year for care.

The study involves the careful recording of events during pregnancy. The mother's genetic and socioeconomic background, past family and medical history, obstetrical and general physical findings are looked into. Laboratory studies include, in addition to the usual, the documentation of infections including those of virus etiology. Events occurring during labor and delivery are recorded. The baby is meticulously evaluated in the nursery as to its pediatric, neurologic and laboratory status. The child is then followed at intervals until he is in school.

The Hopkins group entered the study in 1958 and is now contributing approximately 600 patients per year. A total of 1,065 patients had been enrolled as of March 31, 1960. The oldest children are just approaching their second birthday. The organization for follow-up has been developed to the point where even though 30 per cent of our patients move each year the following percentage of successful follow-up has been achieved.

4th Month—88.2 per cent
8th Month—85.0 per cent
12th Month—86.0 per cent
18th Month—89.0 per cent

A small sample of the kind of information which will become available is apparent from the review of the 226 12th month neurologic examinations completed as of March 31st:

151 children were neurologically normal
14 children were neurologically normal but had other abnormalities
15 children were neurologically abnormal
12 children were neurologically normal but retarded
34 children were neurologically suspicious

The data collected on each patient in the study are fully edited and then forwarded to the central study offices at the NINDB where it is pooled with that from other institutions, coded and processed.

A variety of ancillary studies are being carried on using the same population in the areas of virology, psychology, biochemistry, physiology pathology and audiology, to name only a few.

THE OBSTETRIC ETIOLOGY OF 500 CASES OF CEREBRAL PALSY IN THE NEW YORK AREA

NICHOLSON J. EASTMAN

Dr. Eastman reported a retrospective analysis of the obstetrical background of 500 cases of cerebral palsy in the New York area compared with the obstetrical background of 500 essentially normal children. The investigation was made with the collaboration of Dr. Schuyler G. Kohl, associate professor of obstetrics and gynecology in the State University of New York, and Dr. Jerome Maisel, assistant professor of pediatrics in the same institution. A study of the obstetrical records of the two series of children, the 500 cerebral palsied and the 500 normal, revealed that the two groups were practically identical in respect to the proportion in each of private patients, of white patients, and of primiparas. If trauma played an important role in the etiology of cerebral palsy it might be supposed that the cerebral palsy series would have shown a larger percentage of first-born children than the controls since first labors are more likely to be associated with mechanical difficulties and consequent brain trauma. The present data were not in keeping with such a view since the percentage of first-born in the two series was almost the same.

As was to be expected from previous studies, the percentage of premature births in the cerebral palsy series was extremely high, namely, 30 per cent, as compared with 7.8 per cent in the control group. A particularly noteworthy finding in respect to prematurity in the present data was the high correlation between cerebral palsy and extreme prematurity. Thus, 39 of the cerebral palsied infants had weighed less than 1,500 grams at birth, whereas only two in the normal control series fell into that low weight group. This tendency of very small premature infants to develop cerebral palsy is without adequate explanation and provides a fertile field for investigation especially in prospective studies. The percentage of very large babies was approximately the same in the two series, again an observation suggesting that in present-day obstetrics trauma at delivery may not play as important a part as was formerly thought.

There were two sets of twins in the control series and 26 in the cerebral palsy group. In 24 of the latter series of twins, one twin developed cerebral palsy but in two cases both twins were so affected. Although this high incidence of twinning in the cerebral palsy group suggests that multiple pregnancy *per se* may play a role in the etiology of cerebral palsy, further study of the data indicates plainly that twins are more likely to develop cerebral palsy solely because they are more prone to be born prematurely. Thus, in those deliveries in which both twins were premature, there was no difference in the frequency of multiple pregnancy in the cerebral palsy series and the control group.

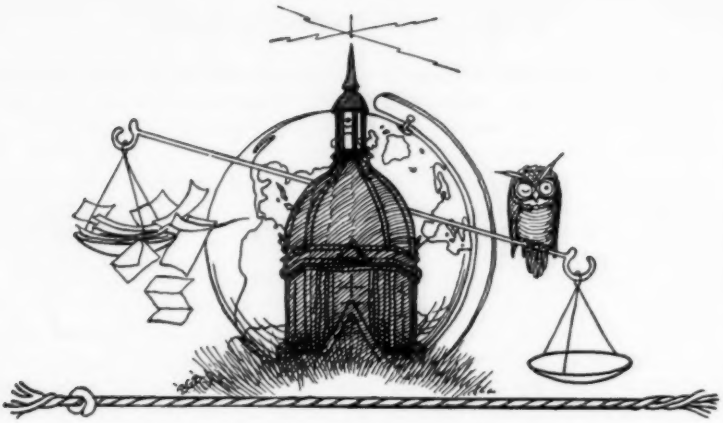
A detailed study of the two sets of twins in which both infants subsequently developed cerebral palsy suggested that a hereditary factor may have been responsible for ataxia in one set while extremely severe attacks of diarrhea, with possible hypernatremia, in the other two infants may possibly have played a causative role.

Although a history of breech delivery was more frequent in the cerebral palsy series, this was apparently related to the fact that a larger proportion of the breech deliveries in the cerebral palsy series were premature. In other words, when prematures were eliminated from consideration, the incidence of breech delivery in the two series was approximately the same.

The role played by intra-uterine anoxia in the etiology of cerebral palsy was demonstrated by the fact that the incidence of abruptio placentae, prolapse of the cord and placenta previa was significantly higher in the cerebral palsy series.

Low forceps delivery, pitocin stimulation of labor, analgesic and anesthetic drugs and duration of labor were noted with about the same frequency in both series, an observation which was interpreted as meaning that these factors played no part in the causation of this disease. Nor was the incidence of elective cesarean section any higher in the normal control series than in the cerebral palsy group. About 5 per cent of the cases of cerebral palsy were attributable to erythroblastosis but in evaluating this figure it must be noted that some of these infants were born before the time when exchange transfusions were prevalent.

In sum, the study shows what can be accomplished in a retrospective study in this area of research but reveals at the same time some of the shortcomings of this type of investigation. On the basis of the data it would appear that in present-day obstetrics trauma in the course of labor and delivery is an infrequent cause of cerebral palsy. Finally, the study not only reaffirms the transcendent role of prematurity in the causation of cerebral palsy but emphasizes particularly the great frequency with which very small premature infants develop this disease.



BOOK REVIEWS

X-ray Technology, 2nd edition. By CHARLES A. JACOBI AND DONALD E. HAGEN. 453 pp., The C. V. Mosby Co., St. Louis, Mo.

This book comprises a survey of the essential elements necessary in a course of training for the X-ray technician. There are chapters on electricity and magnetism, x-ray circuitry and on the elementary physics of radiation. The greater part of the book is devoted to radiographic anatomy and positioning. Most of the material treated here is taken from Merrill's "Atlas of Roentgenographic Positions." The book is terminated with a compact appendix and glossary.

Considering the fact that most of the material in the book is abstracted from other sources which in themselves should be available to the student of X-ray technology, it is difficult to recommend a book of this type as essential, particularly at the price of $10.00.

D. J. TORRANCE

Christopher's Textbook of Surgery, 7th edition. By LOYAL DAVIS. 1551 pp., $17.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

For many years this familiar text has been an outstanding one. A number of changes have been made in the last two editions under its new editorship. The high quality and completeness characteristic of previous editions has been maintained and much valuable information added. The present volume is comprised of contributions from 83 well selected authorities in the United States and Canada. The chapters on Infections, The Abdominal Wall and Peritoneum, Surgery of Peptic Ulcer, The Urinary Tract, Male Reproductive System, The Foot, and Amputations have been completely rewritten by new authors. The final chapter, "Surgical Judgment", is a totally new addition. This section is highly recommended and covers a difficult subject in a praiseworthy manner. A quote by Dr. Charles W. Mayo found in this chapter bears repitition:

> "Surgical judgment differs from surgical skill which implies the quality of technique that, for the most part, is confined to the time a patient actually is undergoing an operation. It is during this period that the quality of judgment should be most dominant over the best of technical skills. This statement is not made with

> any intent to belittle the value of dexterity but rather to place it in proper perspective. . . . The surgeon whom I would select to tend my family or me must first know when not to cut, then when and where to cut, how to cut, and when to stop cutting."

It is this type of consideration which is of particular importance not only to students but to house officers and practitioners of surgery.

The present edition of *Christopher's Textbook of Surgery* has maintained the high standards set by previous ones with the notable addition of recent contributions in the field. It is highly recommended as a companion for medical students as well as a reference source for those engaged in the teaching and practice of surgery.

DAVID C. SABISTON, JR.

Roentgenologic Diagnosis in Ophthalmology. By EDWARD HARTMANN AND EVELYN GILLES. Translated by George Z. Carter. Edited by Conrad Berens. 375 pp., $15.00. J. B. Lippincott Co., Philadelphia, Pa., 1959.

The original work, *The Atlas of Clinical Radiography in Ophthalmology*, was first published in 1936 by the senior author, an ophthalmologist. With the help of the co-author, a radiologist, the text was greatly enlarged and completely re-written. Written from the viewpoint of both the ophthalmologist and radiologist, it remains the only available textbook on this subject. The details of roentgenographic technique are given completely. Routine and specialized roentgenographic projections are described fully, and are well illustrated by line drawings. The entire work is illustrated with numerous X-ray reproductions of good quality. The authors have made frequent use of tomography for the demonstration of both osseous and soft tissue abnormalities. The special techniques for localization of foreign bodies, orbital pneumography, naso-lacrimal contrast visualization, and orbital phlebography are adequately covered. The presentation and case illustrations are excellent for orbital tumors, lesions of the optic foramina, orbital fissures, and sella turcica. Some of the later chapters in the book discuss somewhat superficially lesions of the calvaria, sinuses, teeth, and mastoids, carotid arteriography, and ventriculography. These chapters have been more adequately covered in radiological textbooks on the skull and central nervous system. Nevertheless, this textbook is a valuable contribution to the radiological literature, chiefly because of the authors' extensive experience in this field.

JOHN G. MCAFEE

Regulation of Cell Metabolism. Ciba Foundation Symposium. Edited by G. E. W. WOLSTENHOLME AND CECILIA M. O'CONNOR, 387 pp., $9.50. Little, Brown & Co., Boston, Mass.

During the past two decades great strides have been made in the delineation of intracellular enzymatically catalyzed metabolic pathways. More recently, increasing investigative attention has been directed toward a description of the various mechanisms by which these metabolic processes are regulated.

Biologists, whose experiences are largely derived from multicellular organisms, classically turn to hormones and the nervous system when considering regulatory mechanisms. This symposium, however, is concerned mainly with the factors which act at a more "primitive" level in the cell. Among the topics considered are the meaning of intracellular structure for metabolic regulation, quantitative aspects of the control of oxygen utilization, limiting factors in glycolysis of ascites tumor cells, enzymatic regulation of fermentation in yeast cells, and the control of enzyme synthesis. Sir Hans Kregs served

as conference chairman. Numbered among the contributors were Chance, Dean, Greville, Hinshelwood, Lehninger, Lipmann, Martius, Potter, and others.

This volume contains a record of the proceedings which occurred in July 1958. The formal presentations and discussions have been reported in the manner characteristic of the Ciba Symposia.

ALBERT H. OWENS, JR.

"Allergic" Encephalomyelitis. Edited by MARIAN W. KIES AND ELLSWORTH C. ALVORD, JR. 576 pp., $13.50. Charles C Thomas, Springfield, Ill., 1959.

This book is composed of a series of papers, verbatim discussions, a summary and conclusion presented at a symposium on experimental "allergic" encephalomyelitis and its relation to other diseases of man and animals held at the National Institutes of Health in the autumn of 1957. It, therefore, presents information that was in certain instances out of date at the time of publication. Except for this drawback, which is unfortunate but commonly encountered in an effort of this kind, the book puts forth a wealth of data and interpretative analysis. Perhaps, to some persons, the verbatim transcription of discussions will add an intimacy to the book but to others this does not lend itself well to ease of reading and study.

The symposium is divided into three major sections, including a series of papers on I Clinico-Pathologic Correlations, II Identification of Encephalitogenic Materials, and III Immunologic Aspects. The authoritativeness of the papers is illustrated by the authors' names, but a few of the papers stand out as elegant presentations of a complex and often elusive subject. Among these are the presentations by Raymond D. Adams on "A Comparison of the Morphology of the Human Demyelinative Diseases and Experimental 'Allergic' Encephalomyelitis", Merrill W. Chase on "A Critique of Attempts at Passive Transfer of Sensitivity to Nervous Tissue", and the "Summary and Conclusions" by Ellsworth C. Alvord, Jr.

As is true in many studies, it was difficult to correlate precisely the experimental observations in animals with the clinical diseases of man. In our present state of knowledge it is probably preferable that this correlation not be carried too far. These correlations are compounded in experimental "allergic" encephalomyelitis by the lack of agreement, clearly seen in this book, as to the characterization of the corresponding neurological diseases in man.

L. E. CLUFF

BOOKS RECEIVED FOR REVIEW

Clinical Management of Behavior Disorders in Children, 2nd edition. By HARRY BAKWIN AND RUTH MORRIS BAKWIN. 597 pp., $11.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

Communicable and Infectious Diseases, 4th edition. By FRANKLIN H. TOP. 812 pp., $20.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

From Fish to Philosopher. The Story of Our Internal Environment. Ciba Edition, Revised and Enlarged. By HOMER W. SMITH. 304 pp. Issued by Ciba Pharmaceutical Products, Inc., Summit, N. J. with Permission of Little, Brown & Co., Boston Mass., 1960.

Glaucoma. Transactions of the Fourth Conference, March, 1959. Edited by FRANK W. NEWELL. 257 pp., $8.00. The Josiah Macy, Jr. Foundation Publications, New York, N. Y., 1960.

ORGAN CULTURES OF HUMAN FETAL SKIN FOR THE STUDY OF SKIN DISEASES[1]

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INTRODUCTION

The investigation of many skin diseases is difficult because of the lack of a suitable laboratory model. We therefore have explored factors which permit the maintenance of full thickness human embryonic skin *in vitro* for periods up to six months.

Tissue culture, or the growth of primary cell cultures and of cell lines maintained in continuous culture, has long been used to study the reaction of one cell or of a group of cells to an infecting viral agent. The somewhat related method of organ culture, in which bits of a given type of tissue are grown so that the cells remain in an organized state and preserve their morphological relationships as well as many of their functional activities, has been used in our studies. Fell and others have studied various embryonic tissues (1 to 4), and the interrelationships of endocrine glands in organ cultures, but only recently has this method been applied to basic problems of specificity of infection (5 to 7). It has now been shown that highly malignant tissue grown on organ cultures of a variety of embryo chick organs invades the organs, despite the lack of circulation (8). The effects of a tumor agent on organized salivary gland cultures has also been reported (9).

MATERIALS AND METHODS

Individual skin explants were cultured by a technic similar to that developed at the Strangeways Laboratory and clearly described by Fell (1). A ring of absorbent cotton was saturated with distilled water and placed in a 6 cm. Petri dish. This was used as a base to support a 2.5 cm. watch glass into which the culture medium was placed. To prepare the medium, an extract of 10-day chick embryos was diluted to 50 per cent with Earle's saline solution, and three drops of diluted extract were placed in the watch glass. Ten drops of fresh chicken plasma were then added, and the mixture was stirred with a glass pipet and allowed to clot. It was necessary to obtain the plasma by using siliconed and oiled glassware without anticoagulant, as anticoagulants were found either to be toxic or to produce a clot which was not firm enough to support the explant properly.

Thirty white and negro human fetuses, obtained from spontaneous or therapeutic abor-

[1] Supported by a Grant-in-aid (E-135) from the U. S. Public Health Service.

[2] Research Fellow of the Damon Runyon Memorial Fund for Cancer Research.

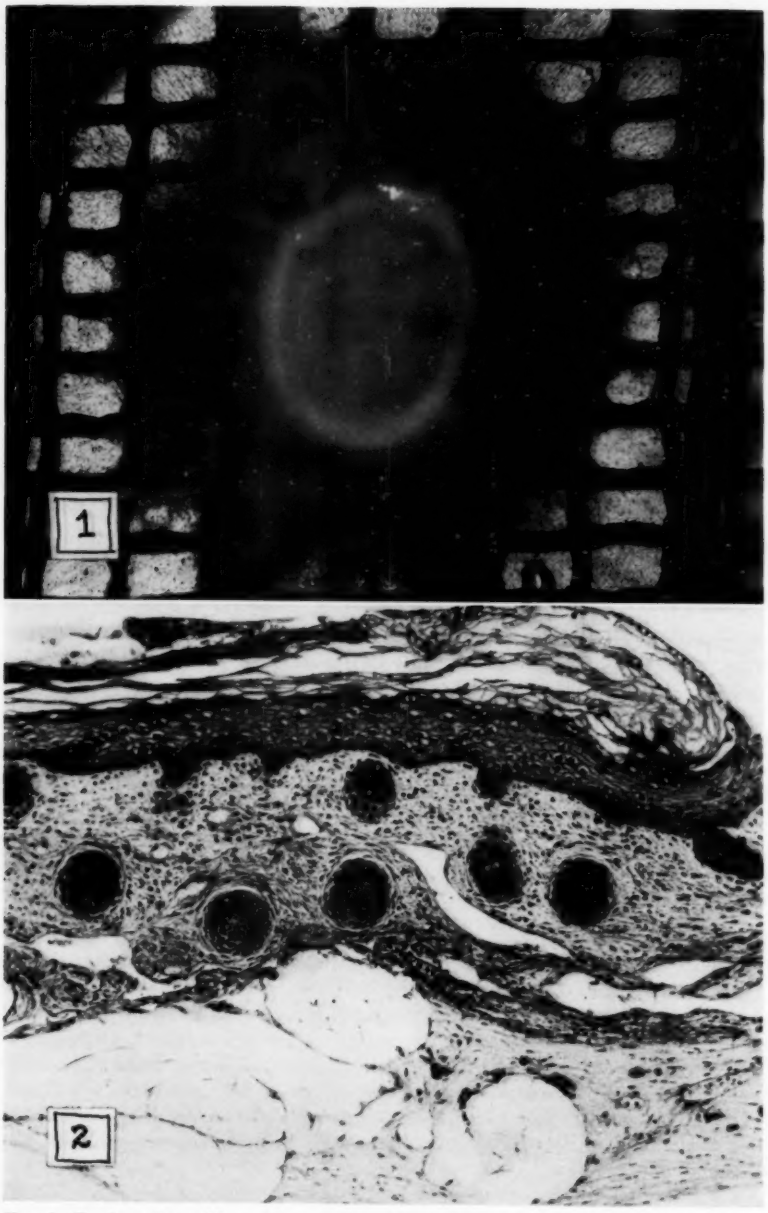


FIG. 1. Explant from 15 cm. fetus, cultured on plasma clot in air, 37°C., for 20 days. × 20.

FIG. 2. Explant after 11 days of culture on plasma clot in air, 37°C. Stained with hematoxylin and eosin. × 150.

tions,[3] were used for organ cultures. Strips of skin were aseptically removed from the trunk and extremities as soon after death as possible. Insofar as feasible, the skin was detached at the lower border of the dermis. It was kept moist with Gey's balanced salt solution and refrigerated at 4°C. until explanted, usually within 6 hours. Other specimens were obtained, also following therapeutic abortions, from Copenhagen, Denmark. These had been rolled in gauze, submerged in Gey's balanced salt solution to which had been added 10 per cent chicken serum, 1000 units per ml. of penicillin and 100 microgm. per ml. of streptomycin. They were air-shipped under refrigeration to Baltimore, and explanted within two to seven days after the original excision (10, 11).

To prepare the skin for explanting, a fragment was placed in a Petri dish, moistened with 1 ml. of Gey's balanced salt solution to which 100 units per ml. of penicillin and 10 microgm. per ml. of streptomycin had been added, and cut with sharp # 11 Bard-Parker blades into one to two millimeter squares. Each square of skin was then placed, stratum corneum up, in the center of a 1 centimeter square of acetone-soluble rayon, which had been washed with distilled water, absolute alcohol and ether, according to the method of Shaffer (12). The rayon bearing the skin was then placed on the surface of a plasma clot, the Petri dish was closed, and the explants incubated at various temperatures between 25°C. and 40°C. The explants were transferred to fresh clots every two to five days; the rayon raft was lifted from the old clot with forceps, and rayon and skin were transferred as a unit.

In some experiments, the glucose level was raised by adding 1 drop of distilled water containing 300 mg. per cent of glucose to the chick embryo extract in the watch glass before addition of the chicken plasma. In another series of experiments, some explants were placed on a medium similar to that used by Blank, *et al.* (13), comprising 0.9 per cent agar in Earle's saline with tris buffer, 30 per cent chicken serum and 10 per cent chick embryo extract. Various explants were placed on media containing 7 per cent human serum, or containing 0.01 per cent neutral red or other vital stains, or were maintained in an atmosphere of 15 per cent CO_2.

Human adult skin, fetal mouse skin, and chick skin were also studied. After designated periods of cultivation the explants were inoculated by inserting a 29 gauge needle through a droplet of virus or tissue suspension into the surface of the explant, or by placing a small fragment of tissue in contact with the edge of the explant.

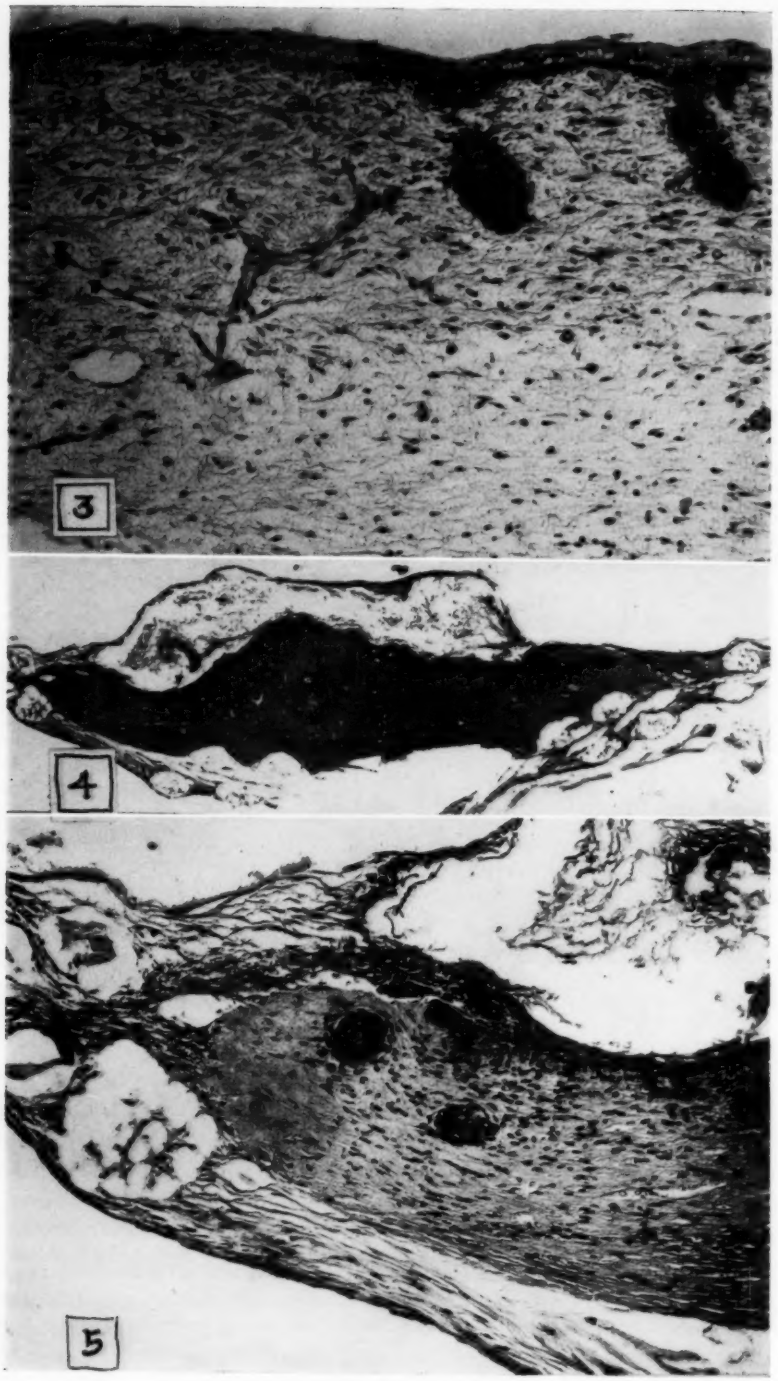
For histological study, the rayon with its attached skin explant was fixed for two hours in one-half strength Zenker's fluid, passed through acetone, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin.

RESULTS

Effect of Age of Donor

Previous studies had indicated that organ cultures of embryonic skin were highly satisfactory, but a more refined spectrum of factors was needed. Therefore a number of adult skin cultures, including twenty adult negro explants, were maintained for three to four weeks. Growth of new tissue appeared only after one to two weeks and remained minimal, and several of the explants degenerated and died during the third and fourth weeks.

[3] We are indebted to Dr. William Hillis and to Dr. H. Anderson of the Dronning Louisa Hospital, Copenhagen, and to the staff of the Department of Obstetrics and Gynecology of Johns Hopkins Hospital for the tissue from therapeutic and spontaneous abortions.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Pigment decreased moderately during the first and second weeks and the skin developed an opaque and unhealthy appearance in many of the explants, taking up a very small amount of vital stain. Culture of skin from fetuses near term was similar to that of adult skin and much less satisfactory than that from younger fetuses. Skin from fetuses younger than 3 months was difficult to handle without trauma and showed poor development after being cultured.

The best results were obtained with skin from 13–26 centimeter fetuses, which corresponds to 12–20 weeks of age. Most of the experiments reported here were carried out on embryos within these limits.

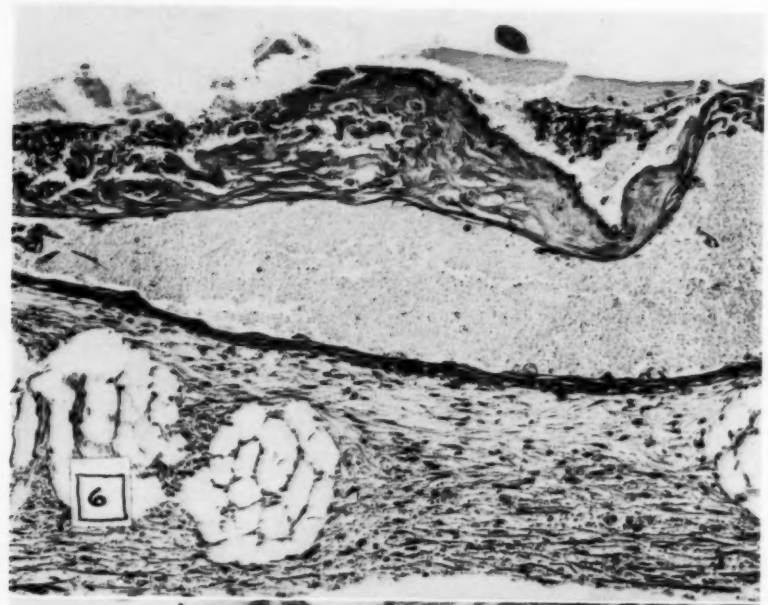
Effect of Temperature and Nutriments

Embryo skin 12–20 weeks of age cultured on embryo extract-chicken plasma clots in air and incubated at 33° to 37°C. showed excellent results. Under these conditions it was found that 302 of 337, or 90 per cent, of the explants remained viable and healthy. Equally good results were obtained with fresh skin and with skin which had been refrigerated for up to 5 days and shipped from Copenhagen. The out-growth of new tissue was usually noted within two or three days after explanting, first appearing as small crescents of cells growing out along the rayon threads. This outgrowth continued for two or three weeks until the original explant, which had meanwhile rounded up and become more opaque, was surrounded by a wide zone of new tissue, much thicker centrally than peripherally (Fig. 1). After one to two months the outgrowth of new tissue ceased and the diameter thereafter remained unchanged, although the periphery continued slowly to become more dense. Some explants remained viable for as long as six months. They remained grossly healthy in appearance, continued to take up vital stains, responded normally to experimental manipulations, and appeared healthy in stained sections. No antibiotics were used after the first explantation. Between 10 per cent and 20 per cent of the explants eventually became contaminated, usually, however, after many weeks. Although the explants from the negro fetuses were sometimes apparently unpigmented at the time of original culture, a large amount of pigment appeared during the first two weeks in the lower epidermis, the upper dermis, and the developing hair follicles and rete ridges, and pigment-

FIG. 3. Skin from 14½ cm. fetus, refrigerated for 58 hours. Stained with hematoxylin and eosin. × 150.

FIG. 4. Explant from same specimen as Figure 3 after 20 days of culture on plasma clot in air, 37°C. Note the development of the epidermis, the thickening of the stratum corneum, the increased density of the dermis and the fibrous new tissue. Stained with hematoxylin and eosin. × 30.

FIG. 5. Higher magnification of a portion of Figure 3. × 150.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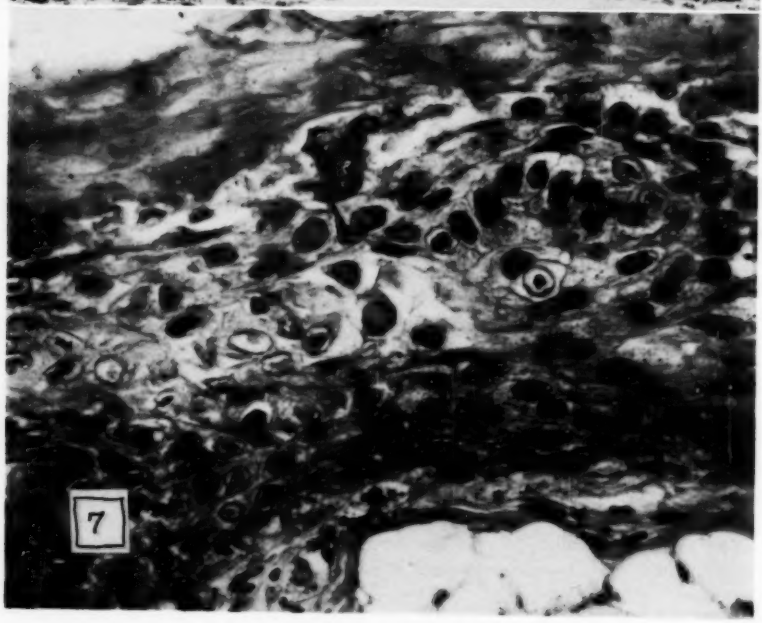
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carrying cells appeared in the newly developed tissue. Skin from most of the white fetuses remained essentially unpigmented.

Microscopic studies showed that the relatively undifferentiated fetal skin continued to develop to some extent after explanting, the dermis thickening and becoming more collagenous and less vascular, the rete ridges and hair follicles increasing in size and in number, the epidermis becoming thicker and more stratified, and the stratum corneum always growing much thicker; the new growth was largely fibrous tissue covered with a very thin epithelial layer (Figs. 2 to 5). Mitotic figures were numerous in the basal epidermis, in the dermis and in the new tissue. This process appeared to reverse after the third month, all layers gradually becoming thinner and less differentiated (Fig. 6).

In experiments in which the temperature of incubation was varied, no difference in response of the skin could be detected between 33°C., 34°C., 35°C. and 37°C. At 25°C. and at 30°C. the skin remained viable, but showed no evidence of growth or of other activity. At 40°C. both viability and longevity were greatly diminished.

Thirty explants were maintained in an atmosphere of 15 per cent CO_2 in air, for several weeks. These showed no apparent difference from controls maintained in air alone.

With the different media studied, it was found that the addition of glucose had no apparent benefit, 41 control explants on clots without glucose doing as well in every way as 39 explants on clots with glucose added. On agar, however, the results were not as good as on plasma, 8 of 40 explants on agar failing to remain viable as contrasted with 2 of 70 controls on the extract-plasma clot. Those explants remaining viable on agar increased in diameter as much as did the controls, but the new growth remained very thin and the original skin also decreased in volume, all layers rapidly becoming thinner. A few explants maintained their viability on agar for as long as two months. Explants which had been maintained on plasma clots for several weeks could then be transferred to agar for as long as two weeks with no gross evidence of degeneration. Explants on agar were much more

FIG. 6. Explant from 24 cm. fetus, refrigerated for 57½ hours before culturing on plasma clot in air, 37°C. Cultured for 129 days. Note thinning and lack of differentiation in the epidermis and dermis, and heavy layer of keratin on surface. The large round defects in the dermis are spaces occupied by rayon threads. Stained with hematoxylin and eosin. × 150.

FIG. 7. Explant from 15 cm. fetus, refrigerated for 58½ hours before culturing on plasma clot in air, 35°C. After 27 days culture was inoculated with Herpes simplex virus and fixed and sectioned three days later. Note intranuclear inclusions and marginated chromatin in most epidermal cells, early vesicle formation, disorganization of basal layer. Stained with hematoxylin and eosin. × 500.

satisfactorily maintained if the Petri dish was placed in a moist rather than in a dry incubator.

Vital stains as a method of determining the viability of explants were investigated, and it was found that better results were obtained with neutral red than with either methylene blue or trypan blue. Either mixing one drop of distilled water containing 0.1 per cent neutral red with the medium, or placing such a drop on the surface of the clot in contact with the explant, allowed the viable cells to incorporate the dye into the cytoplasm, so that characteristic bright red granules were easily seen under low magnification. Such staining usually killed the explant, but occasionally the dye would slowly disappear from the skin and growth and viability would continue.

Viral Inoculations

Inoculation of explants with suspensions of two strains of Herpes simplex virus under a variety of conditions produced intra-nuclear inclusions and other changes characteristic of this disease (Fig. 7). These changes did not develop in explants maintained on media containing human specific antiserum. A culture of negro embryo skin in which pigment was just beginning to appear was inoculated with material from a case of verruca vulgaris; this stimulated the appearance of clubs of pigmented cells extending from the epidermis deeply into the dermis. This activity was lost on passage. Benign and malignant neoplasms, and tissue extracts from these neoplasms, are currently being studied.

SUMMARY AND CONCLUSIONS

Organ cultures of full thickness human skin have been maintained independently of circulation, and in circumstances under which various skin diseases can be studied. The optimal source was from fetuses 13 to 26 centimeters in length. Explants of such skin remained essentially constant in size but showed internal development and differentiation. Optimal temperatures of incubation were 33°C. to 37°C. and optimal nutrition was provided by a clot which consisted of 12 per cent chick embryo extract, 12 per cent saline in 76 per cent chicken plasma. Under these conditions 90 per cent of the explants remained viable and useful for periods up to six months.

Many of the changes which develop in intact normal and infected skin could be studied in serial sections stained with hematoxylin and eosin.

Inoculation with Herpes simplex virus caused characteristic cytologic and histologic changes. It is suggested that such tissues offer a favorable medium for the study of a variety of skin diseases.

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EXPERIMENTAL PSYCHOGENIC HYPERTENSION: BLOOD PRESSURE CHANGES CONDITIONED TO PAINFUL STIMULI (SCHIZOKINESIS)

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Although disturbances in circulation and blood pressure constitute the basis of a great part of clinical medicine as well as a chief cause of death, to what extent blood pressure is subject to psychogenic factors has not been determined. The conditional reflex method, involving as it does precise quantitative measurements of stimulus and response, and of measured stress is well fitted to answer the question of whether or not high blood pressure can become a conditional reflex to individual life experiences, as does the secretion of saliva or the movement of skeletal (voluntary) muscles.

Investigations using Pavlovian methods have indicated that visceral responses such as blood pressure, renal function, and renal hemodynamics may be conditioned. Although the literature contains such reports of visceral conditioning, those results most interesting to medicine have not been known or if known not accepted. Only a few English language physiologic texts mention the possibility of cardiac conditioning, although it has now been a fifth of a century since our original publication on this subject in the dog (1, 2) and that of Beier in the human (3). Wiggers and Best and Taylor are among those texts that discuss the cardiac factors in conditioning (4, 5).

Following the classical research of Pavlov on the salivary glands, many other organs and functions have been conditioned, viz., skin resistance, respiration, heart rate, motor responses of varying complexities, heat regulation, contractions of the spleen, and secretions of the kidneys, pancreas and stomach (6, 7, 8, 9, 10).

In the past two decades, our laboratory has been engaged in the study of the cardiac component of alimentary and defensive conditional reflexes (CRs). In 1934, Gantt and E. C. Andrus attempted to produce a cardiac CR utilizing an adequate cardiac stimulus (electrical excitation of the vagus as the unconditional stimulus). In subsequent work, Gantt, Hoffman, and Dworkin (11), found that an increase in heart rate produced by the eating of food could also be produced by auditory signals after these were

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reinforced with food (12). Continuing studies over the years have demonstrated that the cardiac CR (HR-CR) bears a quantitative relation to what the conditional stimulus (CS) symbolizes: viz., the amount of food or the intensity of pain (13). In a situation involving both cardiac and motor CRs the cardiac CR often appears earlier in the conditioning process than does the motor CR and continues after the motor CR spontaneously disappears or is extinguished. This cleavage between the general, supporting autonomic and the skeleto-motor functions we designate *schizokinesis* (14).

In spite of many researches on circulatory changes in emotional states (15, 16), there have been only a few reports on blood pressure or heart rate as a component of the conditional reflex. Indeed, Watson, the founder of Behaviorism, was unable to demonstrate a change in heart rate in the conditional reflex in human subjects (17).

Beier (3) reported blood pressure as well as cardiac conditioning in three human subjects to a buzzer signalizing exercise on a bicycle ergometer [unconditional stimulus (US)], but there was no quantitative report of conditioned changes in blood pressure. Girden (18), in a later study, measured blood pressure directly from the carotid artery in an anesthesized and subsequently erythroidinized dog, but he did not attempt to produce blood pressure conditional reflexes (BP-CRs) in the non-drug state. The BP rise was established by reinforcing a 250-watt spotlight focussed into the dog's eye with electrical stimulation of the leg. BP-CRs quickly established under erythroidin were absent in the non-drug state.

At the 1953 International Physiological Congress in Montreal, we, as well as Bykov and collaborators, reported BP conditioning, but no systematic papers to our knowledge had then been published to show the development and the differentiation of the blood pressure conditional reflex rise in animals (6).

Since the work described in this article was finished, there have been many important investigations in both Russia and Czechoslovakia on the cardiac conditioning. Korneva and Abdullin of the Institute of Experimental Medicine, Leningrad, using an adequate unconditional stimulus, (olfactory) have formed cardiac conditioning in a number of animals, viz., turtle, frog, rabbit, dove, dog (19).

Fronkova, Slegr and Ehrlich in Prague have performed within the past two to three years important and careful experiments on the conditioning of blood pressure in dogs. Using their ingeniously designed apparatus, they obtain a constant automatically recorded blood pressure showing fluctuations but not absolute measures. Their experiments reveal important adaptations to reserpin and other agents (20).

Many papers have been written on blood pressure as an indicator of emotional states. An early one was by Pavlov (21) in 1879. Noted for his

surgical dexterity, Pavlov, after training a dog to stand quietly, quickly exposed an artery in the leg, inserting a cannula which led off to a manometer. In these studies Pavlov determined the normal blood pressure of dogs, and the effect of various emotional stimuli such as feeding.

Wilhelmj and collaborators showed that marked, sudden elevations in both the diastolic and the systolic BPs were evoked by strangers in the laboratory, previous noises and confusion, alteration of the daily training routine, change of experimenters, and the presence of a female dog in estrus. These writers suggested "that the high degree of restraint and conditioning which are characteristic of the daily existence of the average human subject may eventually result in a similar state of irritability of the vasomotor centers so that minor changes in the daily routine or environment may lead to repeated and excessive acute blood pressure elevations and over a period of years become a factor in the genesis of certain types of hypertension"[3] (22).

George Burch and collaborators at Tulane, using precise measurements, have shown that vasomotor changes are not only susceptible to emotional disturbances, but that they can be learned, i.e. they become conditional reflexes (23).

Since this work was done (1952–53) there has appeared an excellent review of the Russian literature on experimental hypertension, by Simonson and Brozek. Owing to this competent review, no attempt is made to include the many Russian papers on this topic (15).

Procedure

Of the four mongrel dogs in which blood pressure was studied, two of them, Blanket and Whittier, had been used in a previous comparative study of motor and HR-CRs (24). The conditioning procedure consisted of giving three tones and reinforcing each by an electrical stimulus of a different intensity for each tone. Both dogs had formed, before the beginning of the blood pressure measurements, differential motor and heart rate changes specific to the three tones. The blood pressure measurements were begun early in 1953 after the dogs had rested 13 months. Blanket, a male, and Whittier, a female, were about four years old at the beginning of the experiments. The blood pressure experiments for these two animals consisted simply of finding out whether or not a BP-CR had been formed in their earlier training. Neither of the other two dogs (Shep and Browning, males, two years old at the beginning of the blood pressure experiments) had been given any previous CR training. Thus our experiments on blood pressure consisted in 1) measuring the conditioned blood pressure previously formed in Blanket and Whittier, and 2) following the blood pressure changes from the start of work with Shep and Browning. The blood pressure curves in the figures are in general compiled from the daily averages of 10 or more readings for each point on the curve.

The experiments were carried out in several stages.

[3] This modification of the reactivity of the nervous centers would be in our opinion an instance of *autokinesis* (1).

Stage 1 (habituation to new room): The four animals were trained to stand quietly in a sound-shielded room (camera) which separates them from the experimenter and recording apparatus. The animal's movement was restrained by leashing him to a wooden frame; the collar was fastened to an overhanging hook and the left front leg was attached to a turn-table which recorded movement; the extent of movement in the left hind limb was restricted and the blood pressure cuff and microphone were attached thereto (see below). This latter procedure was a new one for all four dogs, and therefore even Blanket and Whittier, previously conditioned, had to have an additional period of habituation. All four dogs learned within two weeks to stand quietly on the table.

Stage 2 (familiarization to attachment of apparatus): The animals were given eight to fifteen daily sessions to extinguish the disturbing effects of inflating the blood pressure cuff (Fig. 1).

Stage 3 (extinction of the orienting response to the tones): A signal which is to be used with some agent such as electrical shock or food does not become a conditional stimulus until reinforcement by the unconditional stimulus. The animal is routinely given those signals which are to be used as signals without reinforcement for 20 trials a day until the orienting responses to these signals are extinguished or reduced to a stable level. This procedure is our pre-conditioning control training or orienting training (25). Browning and Shep required orienting training in the present experiments; they were given repetitions of two tones (256 and 512 cycles), alternately once every two minutes, with a duration of 11 seconds and an intensity comparable to conversational speech.

Whittier and Blanket, who had already been conditioned two years previously, were given extinction trials with three tones of 256, 512, and 1024 frequencies. Each of these signals in the original training routine was reinforced (i.e. followed within a few seconds) by a specific electrical stimulus (US), T256 by a shock of low intensity, T512 by one of intermediate intensity, and T1024 by one of high intensity. Details of this procedure have been reported elsewhere (24, 26).

These two dogs retained the CRs after a 13 month rest, i.e., gave a different HR, motor response amplitude and latency, to each of the three signals at the beginning of the blood pressure measurements. The motor response was a withdrawal of the leg to which the US was applied. There was one alteration of the original procedure, viz., duration of the signals was lengthened from the original 5″ to 11″ to give more time to observe the BP changes.

Stage 4 (conditioning): In Shep and Browning BP was measured from the beginning of training: T512+ was overlapped after 10 seconds by an electrical stimulus lasting 1.8 seconds; T256− was given for 10″ without shock. The US was delivered by a constant amperage stimulator. Blood pressure levels were measured in every training session starting with the familiarization procedure (extinction of orienting reflex) but heart rate and motor responses were recorded only occasionally.

Equipment. Heart rate was measured with an electroencephalograph, and motor responses from the stimulated leg by a lever-device arranged to record resistance changes produced by either vertical or horizontal movement. The horizontal motor component reflected any withdrawal of the body away from the point of electrical stimulation; the vertical component, specific flexion of the leg electrically stimulated.

The blood pressure recording was a modification of a method developed by Allen (27) and Wilhelmj (22). A Brush vibromicrophone taped into position over the femoral artery was used to pick up the arterial sounds. Considerable trial and error experimentation was required to locate the optimal microphone placement for each dog. The microphone was placed just above the ankle joint for Blanket and Browning, and two to three inches below the knee joint for the other two animals. The blood pressure cuff (infant size) was placed with the upper edge overlapping the knee joint. The cuff was prevented from slipping out

of position by a rubber hose running from the cuff to the top of the conditioning frame. The leg was shaven at the site of the microphone placement.

This method is not entirely satisfactory for conditioning studies. Continuous recordings such as are obtained by a needle in the artery are preferable for precision. Daily arterial punctures, however, create many problems, for they damage the arteries, and are often painful since a little blood invariably flows out around the site of the puncture creating a hematoma which in some cases occludes the artery. The electrical recording method of Fronkova *et al.*, though it does not measure absolute pressures, is preferable (20).

The chief disadvantage of the cuff method for conditioning studies is that the latency and the duration of blood pressure changes cannot be studied. Blood pressure readings reported in this paper as a CR were obtained in one or two intervals of time, either during a tone or in the period immediately following in those trials where the US was omitted. Thus, we have no precise record for the latency or the duration of blood pressure changes, aside from noting that the specific blood pressure change to a signal (BP-CR) can occur within 10 seconds and persist for as long as 20 seconds. In Shep and Browning control readings were taken midway between the successive tones, the interval between tones being two minutes; the blood pressure level was nearly always lower than the blood pressure produced by a signal.

Another disadvantage of the cuff method is the fact that every movement of the animal produces a disturbance which tends to obscure the blood pressure sounds (particularly when an animal brushed against the supporting stand). Fortunately, there was a variable period of about five to eight seconds during the first part of the CS in which the animal stood quietly, and another similar period lasting from seven to ten seconds following the US. A final disadvantage of the cuff method is that the diastolic readings were more difficult to hear than the systolic readings.

Despite these limitations the method appeared adequate for the purpose of determining whether blood pressure may be conditioned, and whether the conditional reflex changes are proportional to the intensity of the US. The method is better adapted for studying long term changes in blood pressure than it is for the study of specific CRs of short duration. Some animals, judging by our past studies of tachycardia, should develop either spontaneously or through "conflict", abnormal blood pressure levels with the organic disturbances which are known to accompany this condition.

The reliability of the blood pressure readings which had been demonstrated by Allen (27) and by Wilhelmj (22), was verified in this study by having different people record blood pressure; a nearly perfect agreement was found for our trained observers. The acquired nature of these changes was demonstrated in two ways; 1) by occasionally omitting the US; and 2) by the extinction trials given Whittier and Blanket.

RESULTS

Inflating the cuff initially disturbed all animals including Whittier and Blanket previously given extensive CR training in this same room (Table I). After 8 to 15 daily training sessions the blood pressure decreased to a normal level (Figs. 1 and 2). Ordinarily, we attempted to obtain five readings for both the diastolic and systolic BPs each day. This was not always possible, especially in the beginning sessions, for the dogs became very restless as soon as we began to inflate the cuff. For those days in which we did not secure five readings no range is recorded in Table I. Whether or not

TABLE I

Familiarization BP Levels

Sessions	Whittier		Blanket		Browning		Shep	
	Blood Pressure	Range	Blood Pressure	Range	Blood Pressure	Range	Blood Pressure	Range
1	158/102	20/—	165/102		219/94	90/—	169/—	
2	162/109	8/16	207/153	26/—	177/107		223/166	
3	155/110	22/16	158/113	22/10	153/94	20/12	175/130	
4	125/81	24/22	185/109		130/87	20/10	128/55	
5	134/87	20/24	116/75	24/20	162/82	8/4	164/88	30/10
6	124/78	30/10	182/109		151/87	24/40	168/86	10/8
7	120/84	8/4	134/90	34/26	156/68	22/22	173/97	20/10
8	129/81	20/12	127/82	24/6	155/76	26/8	147/88	10/10
9			128/76	20/20	132/72	24/14	136/74	30/10
10			130/64	18/24	133/69	24/10	142/78	14/10
11					118/68	14/8	142/76	14/10
12					119/65	16/6	136/74	10/16
13							132/70	16/14
14							130/70	10/8
15							128/72	10/8
16								

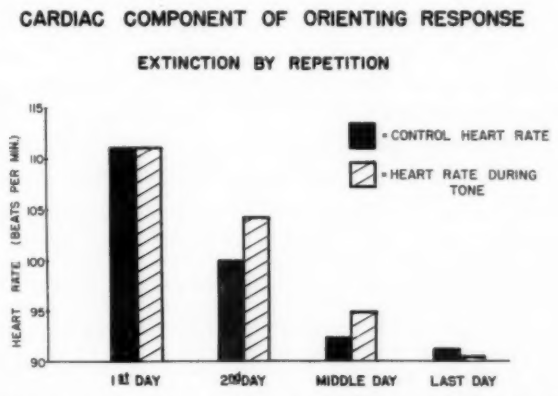

FIG. 1. Cardiac component of orienting response. Figure 1 shows the decrease of the cardiac component in the orienting response to both a new stimulus (tone) and to a new environment for a period of about 3 weeks. When the dog is first brought into the strange environment during the 30 minutes he is there his heart rate is elevated to such an extent that the tone causes no increase. On the 2d day there is less cardiac reaction to the surroundings, and therefore relatively more to the tone. During the 2d and 3d week the cardiac response to both the environment and tone has been extinguished through loss of novelty by repetition. The dog is separated from the person in this experiment as well as in the following figures.

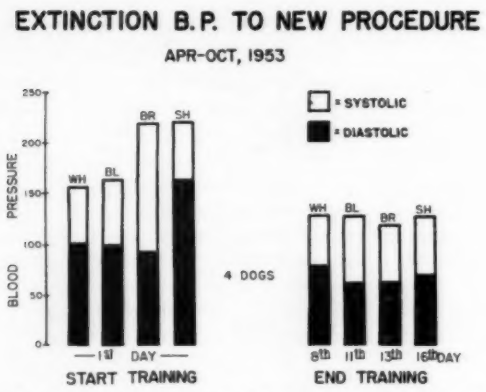

FIG. 2. The blood pressure is measured in 4 dogs when they are first brought into a new environment, and after they have been in the same environment without any experimentation, for 8 to 16 days. In each dog there is a marked lowering of both systolic and diastolic pressure—170–205 systolic and 95–160 diastolic on the first day to 120–130 systolic and 60–80 diastolic on the last day. Blood pressure is recorded automatically without the presence of the person in the room with the dog.

we obtained BP readings, the cuff was always inflated a minimum of 10 times to accustom the dog to the procedure.

In Figure 1 on the first day the HR to the tone was no larger than to the room. This is probably because the orienting reflex to the tone was obscured by that to the strangeness of the total milieu. On subsequent days when the dog became accustomed to the general milieu, the cardiac component of the orienting reflex to the tone becomes apparent.

Tables IIA and IIB present the extinction results for Blanket and Whittier; they show that these two dogs had acquired stable BP-CRs in their

These two tables give for each of five extinction sessions the average systolic and diastolic BP-CRs, the range (the highest value BP-CR minus the lowest BP-CR), the blood pressure level before the beginning of each experiment, the blood pressure level at the termination of each experiment, and the percentage of systolic and diastolic differentiation. The percentage of differentiation refers to the proportion of trials in each session in which the blood pressure level to T 256 was less than the blood pressure level to T 512, the blood pressure to T 512 less than the blood pressue to T 1024, and the blood pressure to T 1024 greater than the blood pressure to T 256. The upper figure in each cell is systolic differentiation and the lower number is diastolic differentiation.

Parallel heart rate and motor CRs were recorded once for each dog during the extinction trials, each animal giving a different heart rate, motor amplitude, and motor latency to the three tones signalizing different intensities of shock. The *heart rate* differentiation tabulated in the manner described above for blood pressure was 81 per cent for both dogs, which was slightly below the systolic blood pressure differentiation.

TABLE IIA

Extinction Trials for Blanket

Date Trials	Tones	BP-CR Levels	BP-CR Range	BP Controls*		Percentage BP-CR Differentiation
				Beginning of Experiment (in-control)	End of Experiment (out-control)	
2 Apr/53	256	145/90	34/20	126/81	142/92	71
1–8	512	146/95	38/12	*12/2*	*20/0*	58
	1024	158/97	30/16			
24 Apr/53	256	181/72	80/20	150/71	180/82	81
10–16	512	178/89	56/20	*20/4*	*20/8*	84
	1024	220/101	60/22			
27 Apr/53	256	185/92	20/20	167/89	188/93	86
17–21	512	206/98	50/10	*10/10*	*10/8*	67
	1024	214/102	40/20			
28 Apr/53	256	161/82	36/22	153/85	146/83	71
22–28	512	170/87	20/10	*10/6*	*10/18*	43
	1024	177/82	50/14			
29 Apr/53**	256	132/82	28/6	131/81	128/82	86
29–35	512	137/83	14/8	*8/2*	*12/6*	57
	1024	147/85	24/8			

* Italicized figures show BP control ranges.

** HR was recorded on this date.

TABLE IIB

Extinction Trials for Whittier

Date Trials	Tones	BP-CR Levels	BP-CR Range	BP Controls*		Percentage BP-CR Differentiation
				Beginning of Experiment (in-control)	End of Experiment (out-control)	
3 Apr/53	256	136/85	16/10	126/80	138/84	65
1–7	512	141/89	36/20	*8/—*	*4/10*	53
	1024	154/92	50/40			
23 Apr/53	256	139/84	20/8	130/81	131/84	76
8–14	512	147/86	30/10	*8/2*	*12/14*	48
	1024	154/89	40/24			
28 Apr/53	256	143/89	26/10	144/90	138/87	81
15–21	512	151/90	26/4	*12/4*	*22/10*	57
	1024	160/93	36/14			
29 Apr/53**	256	143/79	16/12	130/80	143/84	86
22–28	512	156/81	10/12	*4/6*	*6/10*	62
	1024	164/82	26/6			
30 Apr/53	256	129/72	14/2	128/73	126/71	76
29–35	512	134/73	12/2	*12/4*	*8/2*	52
	1024	141/74	28/6			

* Italicized figures show BP control ranges.

** HR record on this date.

previous training which were maintained during the 13-month rest period between the termination of the conditioning procedure and the beginning of the extinction procedure (Figs. 3 and 4).

The *motor CR differentiation* computed on a similar basis was 80 per cent for Blanket and 90 per cent for Whittier. Thus, it appears that in these well trained dogs there was a close correspondence of autonomic and voluntary muscular activity as regards differentiation. Blanket had 500 and Whittier 427 repetitions of the three tones prior to the beginning of the experiments measuring blood pressure.

In the previous conditioning, which terminated 13 months before the beginning of the blood pressure records, there had been formed a definite quantitative change in blood pressure to each of the three tones; this elevated blood pressure was *maintained during the rest period* (Tables IIA and IIB, Figs. 3 and 4). During the five sessions in which the tones were presented without reenforcement, *partial extinction* of the conditioned hypertension occurred, though it had not been extinguished by the previous rest period of 13 months. Thus, in Whittier on 3 April the BP-CRs to the three tones were 136/85, 141/89, and 154/92, but on the 30 April after four additional extinction sessions the corresponding BP-CRs were 129/72, 134/73, and 141/74 (Fig. 3).

Neither Browning nor Shep gave a significant blood pressure or heart rate change to the non-reinforced signals (orienting); in Shep the average of 7 repetitions of the two tones for the first day was 133/77 for T256 and 134/73 for T512; the second day the readings were 127/73 and 123/74 respectively; at the beginning of the experiments before the tones but in the stand the readings were 126/72 and 122/74; at the end of the experiments the readings were 128/74 (first day) and 124/72 (second day). In Browning the average blood pressure for the two tones was 121/73 and 121/71 with the pre-tone control of 122/73.

The comparable heart rates were for Shep 88 and 90 to T512 and T256 respectively, with control, 90; for Browning 65 and 61 to the tones with controls during the preceding 10 seconds of 67 and 60.

In one dog especially (Blanket) the blood pressure showed several consistent changes; 1) an increase which became progressively greater (between 2 April and 24 April) on being returned to the experimental environment (autokinesis);[4] 2) a fall from this peak due to extinction (repetition of signals without shock); 3) a higher level at the end of the experiment than at

[4] Autokinesis is a phenomenon which we have observed especially in neurotic dogs, where the condition gets progressively worse over a period of months or years *after* experimentation has ceased. Our hypothesis is that there is a change in the functions dependent upon a rearrangement of internal relationships independent of the present environment but related to the nervous system changes produced by the past environment (14).

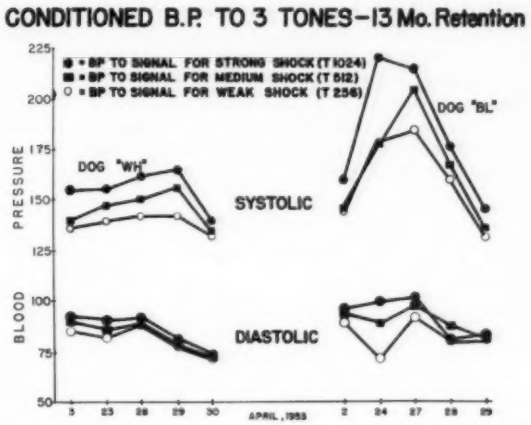

FIG. 3. Conditioned blood pressure to 3 tones. In 2 dogs in which there was formed hypertensive conditional reflexes to 3 signals for different intensities of shock, the hypertension is measured 13 months after the cessation of experimentation. There is seen to be 1) a differentiation to the signals for the 3 intensities of shock, both in diastolic and systolic pressures, and a retention of the BP, 130 to 170 systolic in one dog and 150 to 225 systolic in the other. The hypertension increases instead of diminishes when the dogs are brought back to the stress environment, although there is no repetition of the stress during the month of the record shown (*Autokinesis*).

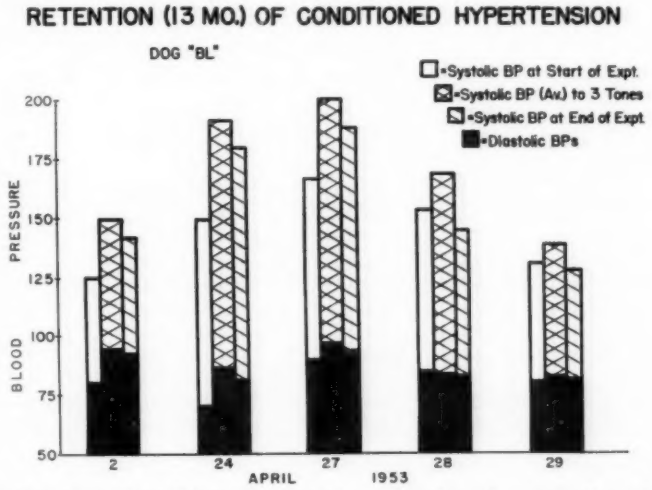

FIG. 4. Retention of conditioned hypertension. This chart shows systolic and diastolic blood pressure 1) at the beginning of the experiment, 2) to the signals for stress (shock), 3) the blood pressure at the end of the experimentation. The dog is separated from the person. No shocks are given during the 29 days shown—the effect is entirely retention of the CR.

The first row for each conditioning session shows the blood pressure and heart rate changes to the nonreinforced T 256, and the second row contains data on the responses to the reinforced T 512. Control blood pressure is the average of readings taken midway between the successive tones, and control heart rate is the average heart rate recorded in the 10-second intervals preceding the onset of tones. In order from left to right, Tables III and IV show the conditioning sessions, tones (column 1), control blood pressure (columns 2 and 3), blood pressure rise produced by tones as a result of reinforcement (columns 4 and 5), control heart rate (HR) (columns 6 and 7), and the HR-CRs (columns 8 and 9).

TABLE III

*BP-CRS in Shep**

Conditioning Session	Tones		Control BP	Control BP Range	BP-CR	BP-CR Range	Control HR	HR Range	HR-CR	HR-CR Range
Session	2	256−			200/—		94	37	102	54
Trials	2–8	512+			200/—		105	47	104	69
Session	3	256−	194/84		214/90					
Trials	9–15	512+	190/86		216/86	20/—				
Session	4	256−	144/64	40/40	146/70	30/20	57	22	55	33
Trials	16–22	512+	140/62	10/24	164/66	50/20	50	23	56	21
Session	6	256−	130/52	20/10	126/54	30/10	62	34	48	22
Trials	29–35	512+	136/50	20/20	144/58	40/10	55	12	56	25
Session	7	256−	158/50	30/20	156/50	20/30				
Trials	36–42	512+	154/46	40/20	174/60	40/30				
Session	9	256−	166/52	30/20	168/50	60/20	57	44	60	20
Trials	46–58	512+	170/50	60/20	180/50	50/20	64	43	64	98
Sesion	12	256−	140/48	32/16	146/52	20/10				
Trials	73–81	512+	148/48	30/6	152/52	50/40				
Session	15	256−	162/64	60/46	184/66	30/40				
Trials	96–102	512+	176/56	30/36	190/62	40/40				
Session	17	256	138/42	36/8	142/48	40/12				
Trials	110–116	512+	140/48	36/6	156/52	46/28				
Session	21	256−	180/50		178/48	18/—				
Trials	138–144	512+	178/48		202/78	20/—				
Session	24	256−	174/48		176/54	32/—	76	98	77	32
Trials	159–165	512+	168/46		182/74	40/—	67	52	93	73

* Range is always based on first seven trials since most sessions consisted of seven trials (seven repetition of the two tones). The range is not tabulated whenever there are less than five readings.

TABLE IV

*BP-CRS Browning**

Conditioning Session	Tones		Control BP	Control BP Range	BP-CR	BP-CR Range	Control HR	HR Range	HR-CR	HR-CR Range
Session	1	256−	174/84	86/30	182/86	108/32	67	20	86	84
Trials	1–7	512+	172/78	88/32	187/86	96/12	68	27	85	86
Session	2	256−	182/100		200/96	80/40	87	39	84	33
Trials	8–14	512+	180/96		202/100	60/40	78	16	84	30
Session	4	256−	188/84		204/88		104	56	120	56
Trials	22–28	512+	178/80		222/88	20/20	89	51	151	62
Session	5	256−	170/82	20/30	194/68	60/40				
Trials	29–35	512+	160/78	24/40	210/76	60/56				
Session	6	256−	176/80		188/86		98	23	110	91
Trials	36–42	512+	178/84	10/10	218/90	40/20	94	44	132	81
Session	13	256−	170/74	24/—	182/80	32/—				
Trials	87–93	512+	178/80	30/—	222/104	50/—				
Session	16	256−	200/90	20/16	204/100	10/24				
Trials	107–113	512+	196/92	10/20	214/102	64/26				
Session	21	256−	158/72	20/12	166/80	12/30				
Trials	142–148	512+	172/80	20/6	182/82	26/—				
Session	24	256−	178/82	18/—	174/88	10/12				
Trials	163–171	512+	178/86		186/94	50/14				
Session	25	256−	169/80	14/—	176/83	28/—	53	26	58	14
Trials	172–179	512+	182/89		190/91	16/8	53	24	61	28

* Range is always based on first seven trials since most sessions consisted of seven trials (seven repetition of the two tones). The range is not tabulated whenever there are less than five readings.

the beginning even though no shock was given during the experiment (Fig. 4. Table IIA). The lack of HR change observed here is unusual, for a dog normally gives a pronounced change in heart rate to the presentation of any mild, novel auditory signal (25).

Tables III and IV give the blood pressure conditioning results for Shep and Browning (Fig. 5). The rise in blood pressure to the shock itself, viz. to the unconditional stimulus, is not recorded in the charts and figures; The figures hence represent the conditional reflex rise, but not the rise to the pain.

In both Shep and Browning there was blood pressure differentiation (comparing the response to T256 with the response to T512) by session 4. In Browning heart rate differentiation appeared about the same time

as blood pressure differentiation, but in Shep heart rate differentiation appeared *after* the beginning of a fairly consistent blood pressure differentiation (usually one or 2 sessions later).

Neither Shep nor Browning developed the stable motor or autonomic CRs seen in other animals in this laboratory. Most animals having auditory stimuli in which only one tone is reinforced learn quickly to discriminate between the two tones, and differentiation after 100 trials is nearly always perfect. The lack of consistency in the present experiment is interesting since neither Shep nor Browning gave an orienting response. The precise relationship between an orienting response and the subsequent CR has not been worked out, although Gliedman has presented evidence to indicate that there is a positive relationship; i.e., the absence of the orienting response predicts difficulty in the formation of the CR (unpublished). Both Shep and Browning were more disturbed (greater restlessness, and variability of responses) by the conditioning procedure than were Whittier and Blanket.

Table V presents data on the differentiation percentages of motor amplitude, latency, heart rate, and systolic blood pressure. Differentiation as used in this paper refers to a contrast between a response to one tone and a response to another tone. In the case of heart rate and blood pressure, one can expect, neglecting the small number of ties, a differentiation of 50 per cent by chance. The chance occurrence of a spontaneous motor movement during a tone is less than this, varying sharply from animal to animal and from one type of conditioning situation to another. In the study described previously in which three electrical stimuli of graded intensities were used (24) it was found that the motor response had a chance occurrence of 20 to 40 per cent.

In Shep, motor amplitude differentiation (height of leg lifting response as recorded by the kymograph) although irregular showed a tendency to increase gradually as a function of the number of conditioning trials. The few sessions in which motor latency and heart rate were studied show a similar irregular trend. Shep gave a differentiation of 83 per cent in the BP-CR at the fourth session and 100 per cent at the seventh session. Following the seventh session the blood pressure differentiation decreased to 57 per cent, and the results from this point on corresponding with the results for the differentiation of the other CR components. Thus, the early split in function between blood pressure differentiation and the differentiation of other responses (schizokinesis) was replaced by parallelism beginning about the ninth conditioning session.

In Browning, motor amplitude differentiation was quickly established

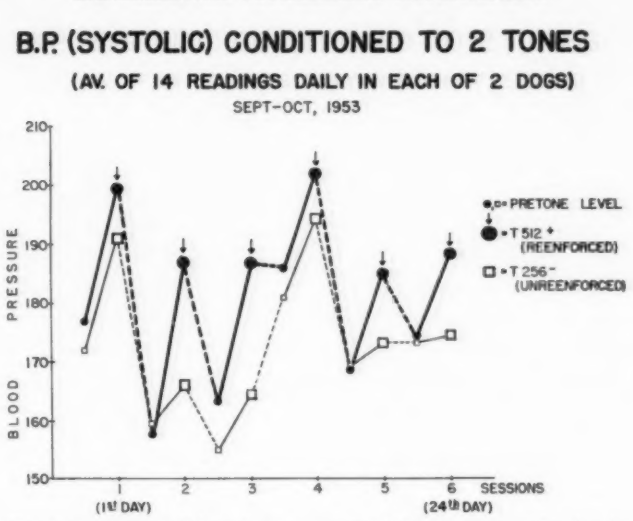

FIG. 5. Blood pressure conditioned to two tones. This chart shows the development of a conditioned systolic blood pressure to a signal for a brief faradic shock to the foot from the beginning of the experimentation. The blood pressure to the actual shock is not shown. Differentiation between the two tones—the excitatory being followed by faradic shock and the inhibitory (T256) without shock—began the first day after a few trials and became gradually better after 24 days. The blood pressure to both signals is elevated, probably as a result of the dog's being in the stress environment.

TABLE V

Comparative Differentiation Motor and Cardiac Conditioning. Per Cent of Responses Greater to T 512+ and to T 256−

Shep					Browning				
Session	% Motor Amplitude	% Shorter Latency	% HR	% Systolic BP	Session	% Motor Amplitude	% Shorter Latency	% HR	% Systolic BP
2	43	57	57	—	1	—	—	57	40
3	14	—	—	—	2	—	—	50	33
4	57	43	57	83	4	100	100	86	100
6	57	57	43	—	5	100	—	—	71
7	57	—	—	100	6	100	57	71	100
9	50	58	67	57	13	0	—	—	100
12	57	—	—	57	16	0	—	—	57
15	86	—	—	67	21	0	—	—	100
17	57	—	—	86	24	53	—	—	86
21	100	—	—	100	25	50	75	75	100
24	71	71	71	71					

and then lost after the sixth conditioning session. In the last three conditioning sessions, the blood pressure differentiation was good, while the motor amplitude differentiation was very poor. This again is a dramatic reflection of the tendency of an animal to show a split (*schizokinesis*) in regard to some body functions. The agreement between blood pressure CRs and heart rate CRs was nearly always greater than the agreement between these autonomic measures and the motor CRs, indicating a pronounced tendency for a split to occur along the lines of general autonomic and more specific voluntary activity (14). This schizokinesis was discovered from our parallel measurements of the heart rate and the salivary and motor conditional reflexes; it has been seen in a number of instances, both normal and especially pathological, e.g., action of morphine in catatonic dogs (28).

DISCUSSION

Blood pressure conditioning was studied to signals for a slightly painful stimulus (electric shock) in four unanesthetized dogs. In two of these the conditioning had been terminated 13 months previously after a year of training; these animals had formed as a result of the training three conditional reflexes of graded intensities with differentiated motor and cardiac components. The three conditional reflexes were directly proportional in magnitude to the three intensities of the unconditional stimuli. The blood pressure responses were not measured at the time of the initial conditioning, but when blood pressure was tested after the rest period of thirteen months it was found that the animals had also formed and retained three blood pressure conditional reflexes that were specific and parallel to the amplitudes of the heart rate increases and the motor conditional reflexes.

In the other two dogs, which had not been previously trained, the blood pressure levels were followed from the beginning of training to two tones, one excitatory (with shock) and the other inhibitory (without shock). To the signal symbolizing shock the blood pressure rose significantly above a control level and above the level obtained to the tone that was not reinforced by shock (inhibitory).

Before beginning either conditioning or extinction trials on these four dogs it was necessary to give them 8 to 15 daily sessions to reduce the disturbing effects of inflating the cuff (familiarization procedure) although the animals had been habituated to the experimental environment. The systolic blood pressure level fell from 167 (median) in the four dogs in the beginning of "familiarization" to 128 at the end of this procedure. While the diastolic readings were not as accurate as the systolic readings, it appeared that the diastolic pressure showed a similar decrement: from over 100 at the beginning of familiarization to 68 at the end of the familiarization procedure.

Thus blood pressure can be conditioned in dogs not only *qualitatively* to

excitatory and inhibitory signals, but specifically, to the *quantitative* significance of the signal. The specific quantitative rise of blood pressure was retained for a 13 months' rest period and was not extinguished by nonreenforcement for a month, i.e., omitting the original stress. In one dog the blood pressure BP-CR continued to rise during the month in spite of there being no repetition of the stress (autokinesis). Blood pressure, a complex autonomic function, is therefore an adjustment not only to the emotional content of the milieu, but to the meaning of the environment to the organism.

There was evidence of a "split" (*schizokinesis*) in the formation of the autonomic and motor responses in the two dogs not previously trained. The systolic blood pressure differentiation was established early in Shep and then paralleled the other components of the conditional reflex (motor amplitude and heart rate). In Browning the blood pressure differentiation was very high in the last several conditioning sessions, but the motor differentiation was little better than chance.

That there is a difference in speed of formation and duration of retention between some of the visceral responses, especially those that are considered to represent emotion, viz. cardiac and respiratory, and the more specific motor ones, has been emphasized in the work since 1944 as evidence of an imperfect adaptation (*schizokinesis*). But its significance and mechanism remain obscure. It was first discovered in normal dogs, but there is some evidence that it may be exaggerated in those dogs under stress—and also in many psychotic patients. Thus in the present experiments the two dogs most conspicuously disturbed were the two animals failing to exhibit a parallelism of conditional reflex components. We recognize the importance of the delicacy of the instruments used for measuring the particular functions being compared; but there is too much evidence for schizokinesis to attribute to it an artifact of instrumentation.

The significance of these experiments is not only that blood pressure like heart rate can be easily conditioned to measured environmental stress imposed during the experiment, but that it is retained for a 13 month rest period—when nothing is being done to the dog. This may be equivalent to chronic hypertension; furthermore the hypertension is experimentally produced by psychogenic stress. As our blood pressure measurements were made only in the environment where the stress was given, we are unable to say whether the hypertension was present outside the experimental milieu.

SUMMARY

1. During habituation in 4 dogs to a new environment and attachment of apparatus, the blood pressure was at first high but fell from about 175 systolic the first day to about 135 on the 9th day.

2. In the first group of dogs used two years previously to form 3 cardiac conditional reflexes to 3 intensities of shock, the blood pressure measured after a 13 month rest was retained and specific to the 3 intensities of shock. In another group of 2 dogs the blood pressure was specific to the excitatory and to the inhibitory signals for pain.

3. The conditioned hypertension was parallel to the conditioned heart rate.

4. The conditioned hypertension was parallel to the motor conditional reflex with certain exceptions: the conditioned hypertension was, like the cardiac conditional reflex, quicker to form and more persistent, thus being present often in the absence of the motor conditional reflex—an evidence of *schizokinesis*.

5. The conditioned hypertension was retained for a 13 month rest period without intervening training, being present immediately when the dog was brought back into the environment where the stress had been given.

6. Although the conditioned hypertension was retained in the long rest period, *it could in one dog be reduced* somewhat by repeating the conditional stimulus without the shock (non-reinforcement), a more efficient way of extinction than simple rest. In another dog the hypertension became exaggerated though there was no repetition of the stress, showing evidence of an internal development (autokinesis).

7. The amplitude of the conditioned hypertension varied according to the individual dog from about 130 average control to limits of 150 to 225 (conditioned hypertension) in the separate dogs.

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COMPONENTS OF BLOOD NECESSARY FOR CLOT RETRACTION[1]

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Platelets are derived from living cells and display metabolic activity (1). The high concentration of adenosine triphosphate in platelets and the rapid disappearance of this substance during coagulation suggest that energy release is involved in the biological function of platelets (2). That platelets are capable of expending energy is shown by their ability to concentrate serotonin and certain other substances against a plasma gradient (3, 4). Viability of erythrocytes, as measured by their survival in the circulation, is known to be dependent upon metabolic activity. It seems not unlikely that survival of platelets *in vivo* is similarly related to continuing metabolic processes. Attempts to control thrombocytopenic bleeding by use of platelets or platelet products were successful only when transfusions of viable platelets were given (5). Experiments in our laboratory have indicated that platelets which were able to cause retraction of clots *in vitro* were frequently, although not invariably, capable of persisting in the circulation after transfusion. In contrast, platelets stored or damaged so that retraction was impaired rapidly disappeared from the circulation (6). These observations substantiate the suggestion (7) that clot retraction may be employed as an *in vitro* test of platelet viability. They also suggest the possibility that clot retraction is dependent upon the metabolic activity of platelets. These considerations have led to a further investigation of the mechanism of clot retraction. The studies to be described were designed to identify those constituents of blood which are essential for the retraction of clots.

METHODS

Glassware with which blood, plasma or platelet suspensions had contact was coated with silicone[3]. The 18-gauge metal needles used for venepuncture were uncoated. Blood from normal human subjects was kept fluid by mixture with either one-fifth volume of acid-citrate-dextrose (ACD) solution, N.I.H. formula A (in experiments involving plasma); one-tenth volume of 3.8 per cent sodium citrate solution (in experiments involving purified fibrinogen);

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[2] Clinical Fellow of the American Cancer Society.

[3] General Electric Silicone SC-87 Dri-Film.

or with 1 to 6 mg. dry disodium salt of ethylenediamine tetra-acetic acid (EDTA) per ml. of blood (in experiments pertaining to effects of EDTA).

Platelet-rich plasma was prepared by centrifugation of freshly drawn venous blood at approximately 50 g for 30 minutes at room temperature. Such plasma usually had a platelet count between 250,000 and 500,000 per cu. mm. Platelet-free plasma was obtained by centrifugation at 22,000 g for 10 minutes. In some studies platelet-free and platelet-rich plasmas were mixed in proportions calculated to yield a desired final platelet count. Platelets were counted by the method of Brecher and Cronkite (8) using a phase-contrast microscope.

Dialyses were performed in a cold room at 4°C using cellulose casing[4] as dialyzing membrane. The bath was either 0.85 per cent sodium chloride solution, or a solution containing 0.85 per cent sodium chloride and 0.3 per cent glucose and was stirred continuously by a magnetic device. The bath volume was at least 300 times that of the plasma undergoing dialysis and was changed three or more times in the course of an experiment.

Suspensions of washed platelets were prepared by centrifugation of fresh platelet-rich plasma at 1500 g for 15 minutes, replacement of the supernatant plasma by barbital buffer at pH 7.6 and dispersion of the platelet sediment with a silicone-coated stirring rod. This procedure was repeated twice and the final volume of buffer was calculated to yield a specific platelet count. Platelets were used within several hours after washing.

Retraction was observed in silicone-coated Pyrex tubes of approximately 11 mm. internal diameter. Immediately after addition of the clotting agent the tubes were incubated at 37°C. Clots were not rimmed, shaken or disturbed in any other way. Retraction of clotted plasma was almost invariably maximal within an hour. Retraction of clots formed from purified fibrinogen sometimes required somewhat longer, but in no case did significant further retraction take place after two hours. Results were recorded photographically, as previously described (7).

Bovine thrombin[5] was dissolved in 0.85 per cent sodium chloride solution to give final concentrations of 1000 units per ml. and 20 units per ml. The less concentrated material was used for experiments involving purified fibrinogen because preliminary studies indicated that the more concentrated thrombin solution inhibited retraction of purified fibrin clots. Bovine fibrinogen[6] was dissolved in barbital buffer at pH 7.6 to make a 2 per cent solution. Serum was obtained from human blood allowed to clot in glass tubes at room temperature. Hydrochloric acid and sodium hydroxide solutions were 0.1 normal or 0.02 normal. The amount of acid or base used to alter pH of the reaction mixture was less than 10 per cent of the final volume of mixture. Barbital buffer at pH 7.6 was made with 2.06 gm. diethyl barbiturate, 2.76 gm. diethyl barbituric acid, 7.30 gm. sodium chloride plus distilled water to make one liter of solution. Phosphate buffer at pH 7.0 was made with 0.67 gm. monobasic potassium phosphate, 5.52 gm. monobasic sodium phosphate, 6.0 gm. sodium chloride, 16 ml. 10 per cent sodium hydroxide plus distilled water to make one liter of solution.

Sugar determinations were done by an enzymatic method (9). Measurements of both bound and unbound calcium of the fibrinogen solution were determined by previously described methods (10).

Determinations of pH were made with a Beckman pH meter, Model H2. Sonic disruption of platelets was produced with a Raytheon Sonic Oscillator, Model S-102A, operating at 50 watts and 9 kilocycles.[7]

[4] Visking Company, Chicago, Illinois.

[5] Kindly supplied by Parke, Davis & Company, Detroit, Michigan.

[6] Obtained through the courtesy of Dr. Walter H. Seegers.

[7] Courtesy of Dr. Robert Schultz, Department of Pathology, University of Maryland, School of Medicine, Baltimore, Maryland.

RESULTS

Retraction of Clots Formed from Platelet-Rich Plasma Containing EDTA

Clots formed by recalcifying platelet-rich EDTA plasma retracted optimally. However, clots formed by the addition of thrombin to platelet-rich EDTA plasma failed to retract unless calcium chloride solution was also added. Non-retractile clots formed by the addition of thrombin to EDTA plasma proceeded to retract normally when calcium chloride solution was carefully layered on the clots as long as two hours after coagulation had occurred. Representative experiments are shown in Figure 1. Platelet-rich plasma prepared from blood containing a high concentration of EDTA (5.0 mg. per ml.) failed to clot following the addition of one part in ten of 0.5 M calcium chloride solution. When this mixture was then clotted with thrombin solution (1000 units per ml.), the resultant clots retracted optimally. Magnesium, strontium or barium chloride was as effective as calcium chloride in enhancing retraction of clots produced from EDTA plasma (Fig. 2). Potassium chloride was not effective.

Retraction of Clots Formed from Platelet-Rich Plasma Containing ACD

A. Effects of dialysis, pH and glucose

Fresh platelet-rich plasma containing ACD retracted optimally when clotted with thrombin. In contrast, platelet-rich ACD plasma dialyzed against an unbuffered saline-glucose bath gradually lost its ability to form retractile clots. After dialysis for 48 hours no retraction occurred on clotting with thrombin. During the period of dialysis the pH of the plasma was

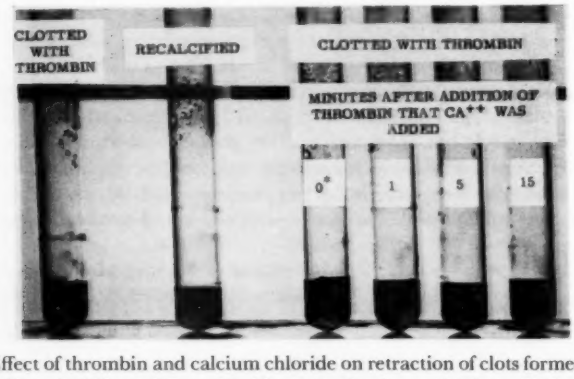

FIG. 1. Effect of thrombin and calcium chloride on retraction of clots formed from EDTA plasma and effect of adding calcium chloride at varying intervals after addition of thrombin. 0.1 ml. thrombin (1000 U. per ml.), and 0.1 ml. calcium chloride (0.1 M.) were added as indicated. All tubes contained 0.9 ml. plasma (260,000 platelets per cu. mm.). Plasma was separated from whole blood containing 1.5 mg. EDTA per ml.

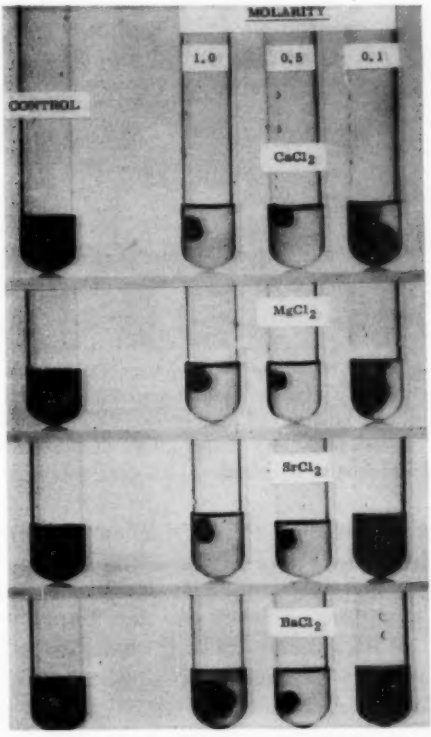

FIG. 2. Effect of several divalent cations on retraction of clots formed from EDTA plasma. 0.1 ml. of divalent cation salt was added as indicated. All tubes contained 0.9 ml. plasma (555,000 platelets per cu. mm.) which was clotted with 0.1 ml. thrombin (1000 U. per ml.) after addition of divalent cation. Plasma was separated from whole blood containing 5 mg. EDTA per ml.

found to approach that of the bath solution, which in these experiments was usually in the range of pH 5.6 to pH 6.0. Clots from plasma so dialyzed retracted normally when as little as one part in ten of platelet-free serum was added, either simultaneously with thrombin or as long as an hour after clotting had occurred. Addition of this small quantity of serum to dialyzed plasma substantially elevated the pH. Serum previously acidified to pH 5.7 with hydrochloric acid was ineffective in raising pH or in restoring clot retraction. Sodium hydroxide or phosphate buffer in the absence of serum not only elevated pH of dialyzed plasma but also restored clot retraction. Representative experiments are shown in Figure 3. Ability to form retractile clots was not lost when platelet-rich plasma was dialyzed for 48 hours against a saline-glucose bath maintained at pH 7.6, whether the pH was

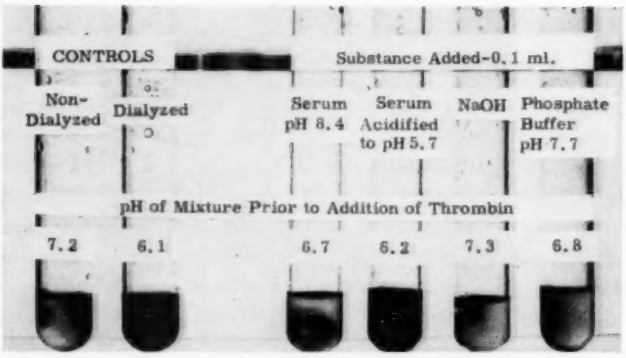

FIG. 3. Relationship of clot retraction to pH change of platelet-rich plasma dialyzed for 48 hours in a non-buffered saline-glucose bath and subsequent restoration of retraction by correction of pH. All tubes contained 0.9 ml. plasma (400,000 platelets per cu. mm.) and were clotted with 0.1 ml. thrombin (1000 U. per ml.). Serum, sodium hydroxide, or buffer were added to dialyzed plasma as indicated.

maintained by use of phosphate buffer or by daily addition of sodium hydroxide to the bath solution.

Aliquots of fresh, undialyzed, platelet-rich plasma were acidified with hydrochloric acid to various pH levels and then clotted with thrombin. Retraction occurred at pH 6.0 and above but was absent at pH 5.6. When plasma acidified to pH 5.6 was mixed with sufficient sodium hydroxide solution to raise pH to 7.3, retraction was restored. Representative results are shown in Figure 4. Clots formed from acidified plasma retracted when sodium hydroxide solution was added as long as two hours after coagulation had been induced.

Platelet-rich plasma dialyzed for 24 hours against a bath of 0.85 per cent

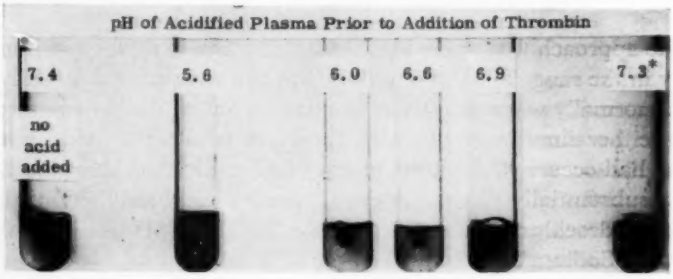

FIG. 4. Effect of acidifying citrated platelet-rich plasma with hydrochloric acid. All tubes contained 0.9 ml. plasma (360,000 platelets per cu. mm.) and were clotted with 0.1 ml. thrombin (1000 U. per ml.) after addition of acid.

* Plasma was acidified to pH 5.6, then titrated to pH 7.3 prior to addition of thrombin.

sodium chloride at pH 7.7 was virtually glucose-free (less than 10 mg. per cent). Such plasma formed clots which retracted poorly. Addition of glucose to this plasma to a final concentration of 200 mg. per cent restored its ability to form normally retractile clots (Fig. 5). Plasma dialyzed for a longer period (48 hours) against saline at pH 7.7 formed clots which did not retract, despite the addition of glucose prior to clotting.

Clots formed from platelet-rich plasma which had been dialyzed for 44 hours against a non-buffered saline-glucose bath did not retract. Serotonin when added in amounts varying between 0.01 mg. and 0.05 mg. per 0.9 ml. of plasma was not effective in restoring retraction. Serotonin was not able to restore retraction to a clot formed from platelet-rich plasma whose glucose content was less than 10 mg. per cent (as a result of 24 hours of dialysis against a saline bath). In fact, serotonin appeared to interfere with the ability of glucose to restore retraction to such plasma (Fig. 5). When added in a concentration ranging between 0.005 and 0.08 mg. per ml. of plasma, serotonin did not restore retraction to clots formed from platelet-free plasma.

In order to determine if dialysis for prolonged periods might remove some other plasma factor essential for retraction, platelet-free plasma was dialyzed for more than four weeks against a large volume of saline-glucose

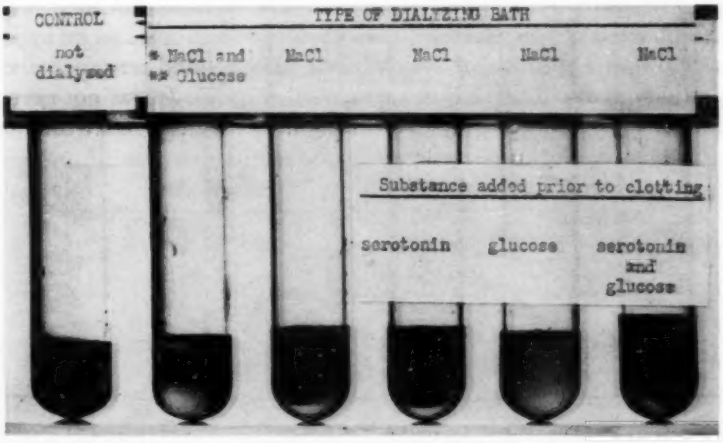

FIG. 5. Relationship of clot retraction to dialysis of platelet-rich plasma for 24 hours against a saline bath with and without added glucose; effect of addition of glucose and serotonin to dialyzed plasma. 0.1 ml. serotonin (3.5 mg. per ml.) and 0.1 ml. glucose (20 mg. per ml.) were added as indicated. All tubes contained 0.9 ml. plasma, adjusted to pH 7.7 at the end of dialysis and were clotted with 0.1 ml. of thrombin (1000 U. per ml.).

* 0.85 per cent sodium chloride.

** 0.3 per cent glucose.

solution. After restoration of pH to physiological range with sodium hydroxide, this plasma was mixed with a suspension of fresh, washed platelets. Retraction occurred normally after clotting was induced with thrombin. Dialysis of thrombin prior to its use as the clotting agent did not alter the results of these experiments.

B. Effects of sonic disruption of platelets

Fresh platelet-rich plasma was exposed to the effects of a sonic tissue disruptor. At intervals aliquots of plasma were removed. Platelet counts were performed on each aliquot and the appearance of the platelets was observed with the phase-contrast microscope as well as in preparations stained with Wright's stain. Portions of each specimen were clotted with thrombin. Complete loss of clot retraction occurred after 60 seconds of exposure, although the platelet count of this sample was 272,000 per cu. mm. and few morphological abnormalities could be detected (Fig. 6). After 5 minutes of exposure to sonic vibrations almost no platelets could be recognized and the preparations contained a large amount of amorphous debris.

Retraction of Clots Formed from Purified Fibrinogen

When purified fibrinogen was dissolved in barbital buffer at pH 7.6 and clotted with thrombin, the resultant clots did not retract. Addition of either washed platelets or serum alone to the fibrinogen solution had no apparent effect. However, addition of both washed platelets and serum resulted in the production of clots which retracted well (Fig. 7). It was not necessary to add calcium. The calcium concentration of the 2 per cent solution of

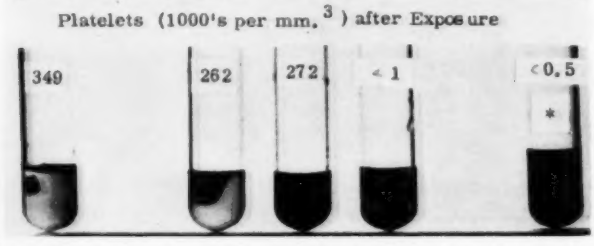

FIG. 6. Effect of sonic vibrations on citrated platelet-rich plasma. 0.1 ml. thrombin (1000 U. per ml.) was added to each tube. All tubes contained 0.9 ml. plasma.

* 0.1 ml. of 2 per cent fibrinogen added after exposure to sound but before addition of thrombin.

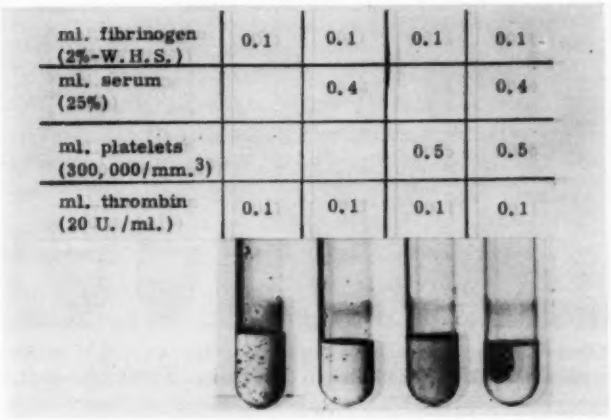

FIG. 7. Substances necessary for retraction of clots formed from purified fibrinogen. Tubes contained barbital buffer pH 7.6 to keep volumes constant.

purified fibrinogen in barbital buffer was 1 mg. per cent, all in the unbound state.

Serum dialyzed for 24 hours against a bath of 0.85 per cent sodium chloride solution lost its ability to enhance retraction in this system. In contrast, serum dialyzed for as long as four weeks against large volumes of 5 per cent glucose solution or sodium chloride solution containing glucose retained its retraction-promoting activity. Furthermore, excellent retraction occurred when glucose was added in place of serum (Fig. 8). Retraction occurred even when the glucose solution was layered on clots which were previously nonretracting (Fig. 9). Mannose, in equimolar concentrations, was as active

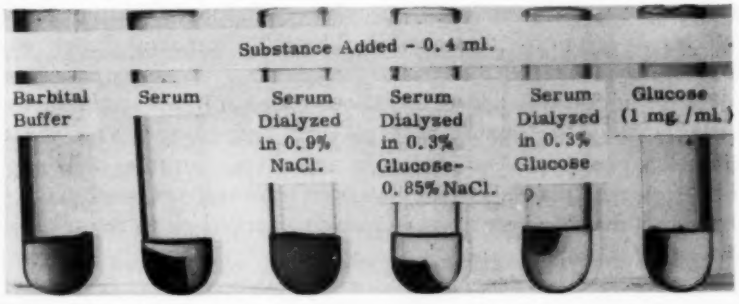
Substance Added - 0.4 ml.
Barbital Buffer
Serum
Serum Dialyzed in 0.9% NaCl.
Serum Dialyzed in 0.3% Glucose-0.85% NaCl.
Serum Dialyzed in 0.3% Glucose
Glucose (1 mg. /ml.)

FIG. 8. Effect of glucose and serum content on retraction of clots formed from purified fibrinogen. All tubes contained 0.1 ml. bovine fibrinogen (2 per cent), 0.5 ml. washed platelets in buffer (400,000 platelets per cu. mm.) and were clotted with 0.1 ml. thrombin (20 U. per ml.).

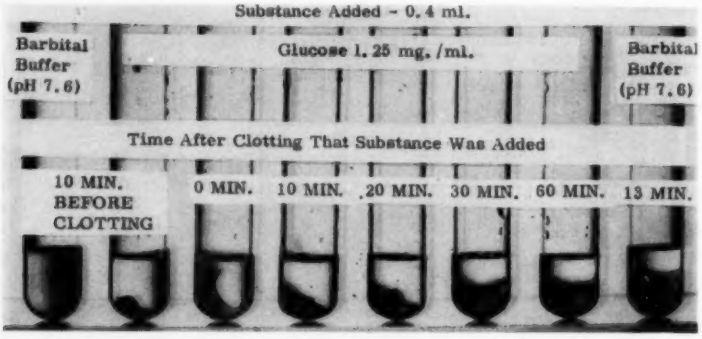

FIG. 9. Effect of adding glucose at various intervals after clotting of purified fibrinogen. All tubes contained 0.1 ml. bovine fibrinogen (2 per cent), 0.5 ml. washed platelets in buffer (400,000 platelets per cu. mm.), and were clotted with 0.1 ml. thrombin (20 U. per ml.).

as glucose; but fructose, d-galactose, d-ribose, sucrose and lactose in equimolar concentrations failed to support retraction. Other substances which were unable to replace glucose in this system (with final mixture concentrations in brackets) were: gum acacia (400 mg. per cent, 800 mg. per cent), bovine albumin (800 mg. per cent), sodium lactate (11 mg. per cent, 55 mg. per cent) pyruvic acid (1.3 mg. per cent, 13 mg. per cent) adenosine (1.5 mg. per cent, 150 mg. per cent) and adenosine-tri-phosphate (11 mg. per cent, 110 mg. per cent, 700 mg. per cent). Substitution of phosphate buffer for barbital buffer did not eliminate the need for added glucose.

In an attempt to determine whether the glucose concentration of the platelets rather than glucose in the surrounding medium was the critical factor, platelets were washed in barbital buffer (pH 7.6) containing glucose in a concentration of 250 mg. per 100 ml. After the second washing the platelets were quickly suspended in buffered fibrinogen solution to which no glucose or serum had been added. Thrombin was immediately added. The resulting clot failed to retract.

Sodium fluoride was added in various concentrations to a buffered fibrinogen solution containing fresh platelets and glucose. This agent inhibited clot retraction when present in a final concentration of 42 mg. per 100 ml. However, platelets which had been transiently exposed to this concentration of fluoride were no longer able to participate in retraction even after several washings.

DISCUSSION

These investigations indicate that a minimum system for clot retraction includes platelets, glucose, divalent cation, fibrinogen and thrombin, interacting at a proper pH. Sodium and chloride were present in the reacting

mixtures in all of the experiments, but the specific role of these ions was not investigated.

Budtz-Olsen (11), on the basis of his own observations and an extensive review of the literature, concluded that intact platelets are necessary for clot retraction. Aged platelets, platelet extracts or fragments, or platelets injured in a variety of ways were ineffective. Observations by many investigators have substantiated the essential role of intact platelets. Furthermore, when platelet-rich plasma was stored, ability to produce retractile clots was lost before an appreciable fall in the platelet count occurred. Platelets which had been frozen and thawed were ineffective in causing retraction of clots unless the platelets were protected during freezing by the presence of glycerol (7).

Several investigators have stated that certain materials derived from platelets or even from other sources can substitute for intact platelets in the production of clot retraction. Fonio (12, 13) and subsequently Morita (14) have reported that retraction could be induced by a specific fraction of platelets disrupted by ultrasonic energy. Other investigators (15, 16, 17) have found that exposure of platelets to ultrasound decreased or eliminated ability to produce retraction. Our experience indicates that platelets, upon exposure to sonic energy under the conditions described, quickly lost ability to participate in retraction and that loss of this function preceded visible morphologic disruption. The type of energy used and the manner of testing, however, were not identical to those described by Fonio.

Serotonin was said to produce retraction of clots formed from platelet-poor plasma (18, 19) but numerous investigators (17, 20, 21, 22, 23) were unable to confirm this report. In our experiments both with plasma and with a purified fibrinogen system, serotonin used in place of platelets produced no retraction. Haverback and his associates (24) observed the occurrence of normal retraction after platelets had been depleted of serotonin by *in vivo* administration of reserpine, an observation confirmed by Hutchison and colleagues (25).

Magalini and Stefanini (26, 27) reported that retraction could be induced in clots formed from platelet-poor plasma by the presence of a factor which was extracted from various tissues by organic solvents. Subsequent work by these investigators has indicated that fibrinolysis was involved in the phenomenon which was observed (28).

The weight of evidence presented to date, therefore, supports the belief that the presence of intact platelets is essential for clot retraction.

As noted previously (20, 29), when purified fibrinogen in a buffered saline solution was clotted by thrombin in the presence of fresh, washed platelets, retraction did not occur; however, retraction was made normal by the addition of small amounts of serum. The present experiments clearly iden-

tify the essential serum factor as glucose. Dialysis of platelet-rich plasma against a saline solution at neutral pH resulted in loss of ability to produce clot retraction, a loss prevented by the addition of glucose to the dialyzing bath or corrected by restoring glucose to the dialyzed plasma. These observations are in accord with those of Bounameaux (30, 31, 32) and of Lüscher (33) who have also found glucose to be essential for clot retraction. Bounameaux (32), however, stated that glucose alone was not as effective as serum unless an "adjuvant" was added. A large number of substances were capable of serving as adjuvants in his experiments. Ballerini and Seegers (17) also reported that glucose and phosphate buffer were more effective than glucose alone in producing retraction in a system consisting of purified fibrinogen, calcium ions, washed platelets in saline and thrombin. We have found that no adjuvant was necessary and that glucose was as effective as serum when the pH of the system was controlled by the use of barbital buffer (Fig. 8). Neither barbital buffer nor phosphate buffer had any effect in the absence of serum or glucose.

The manner in which glucose participates is not known. Of the other sugars tested, only mannose had comparable activity, an observation previously noted by Bounameaux (32). The requirement for glucose in the retraction process implies a metabolic role. Clot retraction is inhibited by sodium fluoride (32), but the mode of action of this agent is difficult to evaluate. Øllgaard (34) noted that clot retracting ability persisted longer when platelets were stored aerobically and in the presence of glucose or fructose. Campbell, Small and Dameshek (1) reported that the clot retraction activity of platelets closely paralleled the activity of oxidase dehydrogenase systems. Our experiments demonstrate that the retraction function of platelets can be restored by addition of glucose after transient removal of this substance. Dialysis of platelet-rich plasma against glucose-free medium for 48 hours, however, resulted in loss of retraction activity which could not be restored by adding glucose. Presumably irreversible changes of the platelets had occurred. Non-retractile clots formed in the absence of glucose were observed to retract when glucose was added as long as several hours after clotting had occurred. This observation indicates that the essential effect of glucose is not necessarily exerted at the time of clot formation and is therefore not concerned with the manner of deposition of fibrin strands.

Previous studies (11, 29) have demonstrated that clot retraction occurs within a relatively wide range of pH, but our experiments show that retraction is inhibited when pH is reduced to approximately pH 6.0 or less (35). This effect of pH has also been noted by Ballerini and Seegers (17) and by Benthaus (36). Distilled water becomes more acid on exposure to the air. When platelet-rich plasma was dialyzed against unbuffered solutions of sodium chloride and glucose, the buffers of the plasma were removed and

the plasma acquired the low pH of the dialyzing medium. Such acidified plasma yielded clots which did not retract unless the pH was elevated. The retraction-promoting effect of serum on clots formed from plasma dialyzed in this way could be attributed to the rise in pH produced by the addition of serum. Clots formed from acidified plasma could be made to retract by elevation of pH as long as several hours after clotting had occurred. As in the case of glucose, therefore, the essential role of pH in retraction appears not to be related to the manner in which the clot is formed.

Our experiments provide no evidence that dialyzable substances other than glucose are essential for retraction when the dialyzing medium consists of a solution of sodium chloride which has been buffered to prevent pH change. The possibility that the thrombin solution employed to form clots was contributing a necessary dialyzable component was excluded by dialysis of the thrombin prior to its use in some of these studies. The nature of the dialyzable factor described by Fenichel and Seegers (18, 19) remains obscure.

The observation that clots formed from plasma containing EDTA did not retract unless a divalent cation was added is in agreement with the report of Zucker and Borrelli (37), who have suggested that magnesium rather than calcium is the ion functioning under physiological circumstances. Our experiments confirm the belief, first expressed by Budtz-Olsen (11), that a concentration of calcium too low to permit coagulation of plasma will support excellent retraction when clotting is produced with thrombin. The essential divalent cation could not be removed by prolonged dialysis. Retraction occurred in the purified fibrinogen system with washed platelets prepared from citrated plasma, although divalent cations were not added. The fibrinogen used supplied 0.001 mg. of calcium ion to each ml. of reaction mixture. The amounts of calcium provided by the washed platelets and the thrombin were not measured. Therefore, the ions chelated by EDTA may well be closely bound to the platelets and not removable by ordinary washing or by citrate. The presence of divalent cation is not required at the time of clot-formation, for its addition hours after clotting of EDTA plasma by thrombin permitted previously non-retractile clots to undergo retraction.

It seems likely that clot retraction is an expression of a complex biological function of platelets acting as cellular structures. "Viscous metamorphosis" appears to be a closely related phenomenon (20, 32, 38). The biochemical processes which provide the necessary energy may be dependent upon viability of platelets. While this latter point has not been proved, there is evidence that platelets irreversibly damaged to the extent that retraction function is lost are nonviable, i.e. will not remain in the circulation *in vivo*. Presumably glucose, divalent cations and an appropriate pH are required if the biochemical reactions leading to retraction are to proceed. The im-

portance of fibrinogen in the retraction process is not known. Its role may be a passive one. Lüscher has described retraction of platelet masses in the absence of fibrinogen (20). Bettex-Galland and Lüscher (39) have reported extraction of an actomyosin-like protein from human thrombocytes, but this material has not at present been related to clot retraction.

The physiological significance of clot retraction has not been determined. Budtz-Olsen (11) doubted that retraction of clots plays a significant role in hemostasis. On the other hand, a hemorrhagic disorder has been encountered in several patients whose only demonstrable disorder was a qualitative platelet defect characterized by absence or deficiency of clot retraction function (14, 40, 41). Of the various activities of platelets which can be measured *in vitro*, ability to produce clot retraction correlates best with viability of platelets *in vivo*. Accordingly, measurement of retraction function may be employed as a useful test of the possible adequacy of various procedures designed for storage or preservation of platelets.

SUMMARY

1. Clots formed from purified fibrinogen by the action of thrombin did not retract unless both platelets and glucose (or mannose) were also present. Glucose was also found to be of importance in the retraction of clotted platelet-rich plasma.

2. Clot retraction of plasma did not occur below a certain critical pH which appeared to be between pH 5.5 and pH 6.0.

3. No dialyzable plasma factor necessary for clot retraction could be demonstrated when pH change and loss of glucose were prevented.

4. EDTA plasma failed to retract when clotted with thrombin but retracted well when calcium chloride was also present. Strontium, barium, or magnesium salts could substitute for calcium.

5. When added within an hour after formation of the clot, the addition of glucose, calcium ion, or hydroxy-ion to clots deficient in these substances initiated retraction.

6. The implications of these findings and various other aspects of clot retraction are discussed.

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CATECHOLAMINE AND 17-HYDROXYCORTICOSTEROID OUTPUT IN DOGS WITH TRANSPLANTED ADRENAL GLANDS

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In 1939 Levy and Blalock (1) described a method for transplanting the adrenal gland of the dog with reestablishment of its blood supply. The left adrenal gland and kidney were transplanted *en bloc* to the neck of the animal. Since blood vessels of the left adrenal gland and kidney intercommunicate about the renal artery and vein in 7 out of 10 dogs (2), the blood supply of both organs was reestablished by suturing the renal artery to the left common carotid artery and renal vein to the left external jugular vein. After a week or longer the kidney was excised. The opposite adrenal gland was removed before or after the transplantation procedure. One of the values of the method is that the external jugular vein lies just beneath the skin of the neck. It can be punctured for sampling of blood which drains exclusively from adrenal tissue which is the only viable adrenal tissue remaining in the animal.

At the time of the original experiments by Levy and Blalock, efforts were made to demonstrate epinephrine elaboration by the transplanted medullary tissue, using histological and bioassay technics. Epinephrine output was not definitely demonstrated, although some of the medullary cells appeared normal on microscopic examination. On the other hand, the survival of dogs with one adrenal transplanted and the opposite adrenal removed was good evidence that cortical steroid hormone was being produced by the transplant. Dogs without adrenal cortical tissue usually die within about five days unless they receive adrenal cortical replacement therapy.

The more recent availability of chemical methods for plasma catecholamine and corticoid determination has made it possible to test directly whether the medullary tissue in these dogs is functional and to quantitate the 17-hydroxycorticosteroid output.

METHODS AND RESULTS

Five dogs were studied in the resting, unanesthetized state. Their weight ranged from 14 to 20 kilograms. All dogs had lived in generally good health for four years or more following left adrenal transplantation and right adrenalectomy. Blood was withdrawn by needle puncture from the external jugular vein just below the palpable adrenal transplant. Femoral venous blood was simultaneously sampled for comparison of peripheral venous and adrenal

venous hormone concentration. The blood was collected in heparinized syringes and centrifuged immediately for separation of plasma. The plasma was frozen. Hormone determinations were made within a week.

Catecholamine measurements were made by the method of Weil-Malherbe and Bone (3).

Measurements of plasma 17-hydroxycorticosteroids (the dog produces cortisol mainly) were performed by the method of Nelson and Samuels (4).

Catecholamine and Corticoid Values in Resting Unanesthetized Adrenalectomized Dogs with One Adrenal Gland Transplanted to the Neck

Dog No.	Plasma Catecholamine Concentration micrograms per liter		Plasma Corticoid Concentration micrograms per cent	
	Femoral Venous	Adrenal Venous	Femoral Venous	Adrenal Venous
601				320.0
603	9	12	0.0	162.2
605				343.5
609	8	8	0.3	345.7
610	8	0	0.0	241.4
Normal Values of Method	8–16		0–3	

DISCUSSION

The failure to find a higher catecholamine concentration in the adrenal venous blood than in the femoral venous blood demonstrates that the medullae of the transplanted adrenals are not functional. Osmic acid stains for granules in the medullary cells, blood sugar determinations after massage of the transplanted gland, and bioassay using rabbit small intestinal muscle activity as an index had previously indicated that the adrenal medullary function was lacking. The glands of the various dogs were without blood supply for 29 to 42 minutes during the transplantation. The loss of the medullary ganglionic cellular activity is not surprising, since this type of cell has an anoxic limitation of less than 15 minutes at 37°C.

The 17-hydroxycorticosteroid concentration in the adrenal venous blood of all five dogs was many-fold higher than that in the femoral venous blood. The adequacy of the corticoid output had been previously indicated by the survival of the animals and by the observation that one female withstood the stress of pregnancy from which she produced a normal offspring.

This preparation provides a convenient method for the study of the effect of other hormones, drugs and various interventions upon the adrenal cortex. The effects of epinephrine infusion, acetylcholine infusion, and respiratory anoxia will be reported later.

SUMMARY

It had previously been demonstrated that the left adrenal gland of the dog could be autotransplanted to the neck with reestablishment of its blood

supply. Chemical studies reported here confirm the corticosteroid hormone output of such transplanted adrenal glands and demonstrate the lack of catecholamine output. The adrenal cortex survives functionally; the medulla does not.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. Gustave Freeman and Mrs. Margaret G. Filbert for their assistance with the catecholamine determinations.

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INACTIVATION OF T2r+ BACTERIOPHAGE BY LYSOZYME[1]

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INTRODUCTION

During the course of studies of the antiviral activity of certain components of normal animal tissues, it was found that purified T2r+ coliphage was consistently inactivated by crystalline egg-white lysozyme. The present paper describes our studies of the conditions for inactivation of T2r+ by lysozyme, the influence of purification of T2r+ on its susceptibility to lysozyme, and the relation of the phage-inactivating and bacteriolytic properties of lysozyme. Also described are additional experiments designed to determine the spectrum of activity of lysozyme and histone against a series of coliphages.

MATERIALS AND METHODS

Bacteriophages. Members of the T series of coliphage were obtained from Dr. Roger M. Herriott, Johns Hopkins University School of Hygiene and Public Health. To prepare crude phage lysates, bacteriophages were mixed with *Escherichia coli* strain B actively multiplying in trypticase soy broth (1) or a synthetic medium (2). Purified T2r+ coliphage, which was used in most experiments, was prepared by the method of Herriott and Barlow (2).

Titration of Bacteriophage. One ml. aliquots of serial decimal dilutions of bacteriophage suspension were mixed with 9 ml. of liquid "suspending" agar[4] (46°C.) containing 10^7 *E. coli* per ml. *E. coli* were obtained from a 4 hour culture in trypticase soy broth. Two ml. of the phage—*E. coli* mixture in "suspending" agar were then spread on a layer of firm "plating" agar[5] in a Petri dish. After the layer of "suspending" agar had solidified at room temperature, the plates were inverted and incubated at 37°C. for 18 hours. The number of

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[4] To prepare "suspending" agar, 6 mg. Bacto agar (Difco), 10 gm. Bacto tryptone (Difco), 8 gm. sodium chloride, 2 gm. sodium citrate and 3 gm. of glucose were dissolved in 1 liter of distilled water and autoclaved at 15 pounds for 15 minutes.

[5] "Plating" agar was prepared by dissolving 10 gm. Bacto agar (Difco) 13 gm. Bacto tryptone (Difco), 8 gm. sodium chloride, 2 gm. sodium citrate and 1.3 gm. glucose in 1 liter of distilled water. Sterilization was accomplished by autoclaving at 15 pounds pressure for 15 minutes.

plaque forming units (p.f.u.) in the original suspension was calculated from the number of plaques observed at each dilution.

Method for Determining Phage Inactivation. One-half ml. of a solution of the substance to be tested and 0.5 ml. of bacteriophage suspension were mixed in tubes in an ice bath and then incubated at 37°C. for one hour. The diluent for the inactivating agent and for bacteriophage was sodium phosphate buffer (0.1 M, pH 6.5) containing 1 mg. per ml. of human serum albumin. Phage incubated with buffer was used as a control. After incubation the tubes were returned to the ice bath, and 5 ml. of cold 0.15 M NaCl solution was added to stop the reaction. The number of p.f.u. in each mixture was determined as described in the preceding paragraph.

Method for Determining Bacteriolytic Activity. The bacteriolytic activity of lysozyme was determined by the rate of lysis of turbid bacterial suspensions. Cell walls of irradiated *Micrococcus lysodeikticus* ("lysozyme substrate"—Difco) were diluted in a phosphate buffer of pH 6.2 ("lysozyme buffer"— Difco) to a concentration of 0.5 mg. per ml. The lysozyme solutions to be tested were diluted in buffer to contain an estimated 0.2 to 0.8 mg. per ml. Standard solutions were prepared by dissolving weighed amounts of lysozyme in buffer.

Four ml. of substrate and 4 ml. of standard or test solution were mixed in a calibrated tube and readings of optical density were made at exactly 5, 10, 15, and 20 minutes after mixing. Optical density was measured in a Bausch and Lomb "Spectronic 20" spectrophotometer set at a wavelength of 540 mμ. All tests were carried out at 34°C. and each of the reactants was allowed to reach this temperature before mixing.

The turbidity of the lysozyme-containing mixtures decreased logarithmically with time and the rate of clearing was proportional to the concentration of lysozyme. The concentration of lysozyme in the test solution was determined by comparing the rate of lysis observed with the rate of lysis in the tubes containing known amounts of lysozyme.

Other Reagents. Crystalline egg-white *lysozyme* was obtained from the Difco Corporation, Detroit, Michigan, and Armour and Company, Chicago, Illinois. Lysozyme from both sources was almost identical in bacteriolytic and phage-inactivating activities. However, the Armour preparation was more readily dissolved in buffer and was used in most experiments. The *human serum albumin*, used in buffers as a stabilizing agent, was Cohn Fraction V prepared by Squibb and Co., and kindly supplied by the American Red Cross. A concentration of albumin of 1 mg. per ml. prevented inactivation of T2r+ by vigorous shaking or by buffers of low molarity (0.01 M). *Deoxyribonucleic acid*, *papain*, and calf thymus *histone* were obtained from Worthington Biochemical Corporation, Freehold, N. J., and *E. coli endotoxin* and *trypsin* were prepared by the Difco Company.

RESULTS

Inactivation of Purified T2r+ Coliphage

The Influence of Concentration of Lysozyme and Bacteriophage

Coliphage suspensions containing 10^4, 10^5, 10^6, or 10^7 p.f.u. per ml. were mixed with graded doses of lysozyme ranging from 5 to 350 microgm. per ml. After incubation of mixtures (pH 6.5, 0.1 M) at 37°C. for 1 hour, the reaction was stopped and the number of residual p.f.u. was determined.

The degree of inactivation was not influenced by a thousandfold changes in concentration of bacteriophage. In repeated experiments it was shown

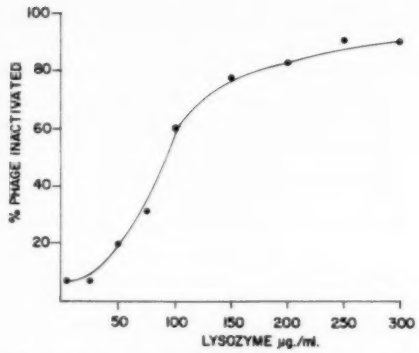

FIG. 1. Inactivation of purified T2r+ coliphage (10^4 p.f.u. per ml.) by graded doses of lysozyme.

that a given amount of lysozyme inactivated an approximately constant percentage of 10^4, 10^5, 10^6 or 10^7 phage particles.

As shown in Figure 1, the degree of inactivation of T2 did not increase linearly as the concentration of lysozyme was increased. Under the conditions of this experiment, maximum inactivation (90 to 95 per cent) was obtained with 250 microgm. per ml. lysozyme and additional inactivation was not achieved by increasing the lysozyme concentration fourfold.

By applying the linear transformation of von Krogh (3) to the dose-response data it was possible to obtain a straight line which allowed an accurate determination of the concentration of lysozyme inactivating 50 per cent of the bacteriophage. The straight line function was obtained by plotting the $\log_{10}$ of the ratio

$$\frac{\text{Percentage of phage inactivated}}{1 - \text{Percentage of phage inactivated}}$$

as a function of $\log_{10}$ of the concentration of lysozyme. Using the data presented in Figure 1, it can be calculated that in 0.1 M sodium phosphate buffer (pH 6.5) 94 microgm. per ml. of lysozyme inactivated 50 per cent of the bacteriophage after incubation for 1 hour at 37°C.

Effect of Temperature, Molarity, and pH

Phage inactivation by lysozyme was markedly influenced by temperature, molarity, and pH of the reaction mixtures.

As shown in Figure 2, maximum inactivation (97 per cent) of 10^4 p.f.u. by 500 microgm. per ml. lysozyme was achieved at 37°C. and raising the temperature to 46°C. did not further increase the amount of phage inactivated. At 4° or 22°C. the reaction proceded slowly with only 40 per cent inactivation of phage after 1 hour.

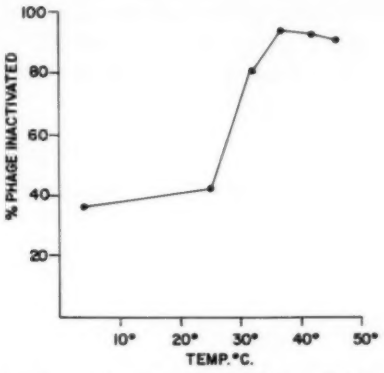

FIG. 2. Effect of temperature on inactivation of T2r+ coliphage (10^4 p.f.u. per ml.) by lysozyme (500 microgm. per ml.)

Inactivation was most marked when the phage-lysozyme reaction was carried out in buffers of low molarity. In these experiments, the diluent for phage (10^4 p.f.u.) and lysozyme was sodium phosphate buffer (pH 6.5) and the reaction was carried out at a constant temperature of 37°C. for 1 hour. Fifty per cent of the phage was inactivated by 24 microgm. per ml. lysozyme in 0.01 M buffer, and by 10 microgm. per ml. in 0.05 M buffer. As the molarity was increased, a larger amount of lysozyme was required for inactivation of 50 per cent of the bacteriophage (0.1 M – 94 microgm. per ml.; 0.2 M – 200 microgm. per ml.).

The effect of pH of the reaction mixture on inactivation of 10^4 p.f.u. by 500 microgm. per ml. lysozyme is shown in Figure 3. In these experiments, molarity and temperature were maintained at 0.1 and 37°C. and the dura-

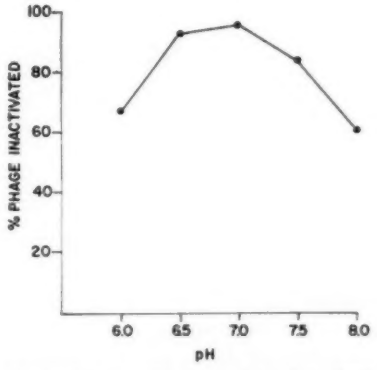

FIG. 3. Effect of pH of reaction mixture on inactivation of T2r+ coliphage (10^4 p.f.u. per ml.) by lysozyme (500 microgm. per ml.)

tion of incubation was 1 hour. Maximum inactivation of phage by lysozyme occurred at pH 6.5 to 7.0. Observations could not be made at pH values below 6.0 or above 8.0 because significant loss of phage activity occurred in control suspensions containing no lysozyme.

Time Course of Inactivation

To study the rate of inactivation, suspensions of bacteriophage and lysozyme previously warmed to 37°C. were mixed; aliquots of the mixture were taken immediately and at frequent intervals during incubation at 37°C. for enumeration of p.f.u. Lysozyme and phage were suspended in sodium phosphate buffer (0.1 M, pH 6.5) containing 1 mg. per ml. of serum albumin.

Inactivation of purified T2 phage by lysozyme did not proceed logarithmically. As shown in Figure 4A, the reaction of 10^4 p.f.u. with 500 microgm. per ml. lysozyme proceeded rapidly until about 80 per cent of the phage was inactivated, but after 20 minutes the rate of the reaction decreased sharply. Although a reaction of the first order was excluded by these data, the possibility of a second order reaction was suggested by the fact that a straight line could be obtained if the reciprocal of the per cent surviving phage was plotted on a time scale (Fig. 4B) (4).

Additional experiments of similar design were performed to determine the influence of concentrations of phage and lysozyme on the rate of inactivation. The *rate* of the reaction was slowed considerably by two or fourfold decrease in lysozyme concentration (Fig. 5) but was not affected by a hundredfold change in phage concentration (Fig. 6). Similar kinetic features have been noted for inactivation of certain coliphages by antibody (6, 7), ribonuclease (8), and protamine (9).

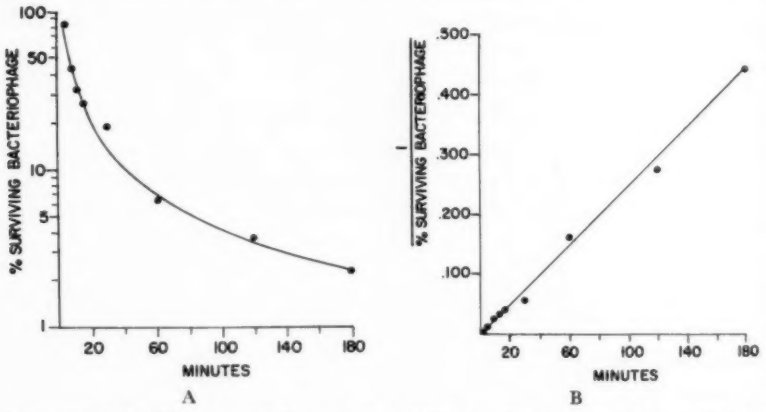

FIG. 4. A and B. Time course of inactivation of T2r+ coliphage (10^4 p.f.u. per ml.) by lysozyme (500 microgm. per ml.).

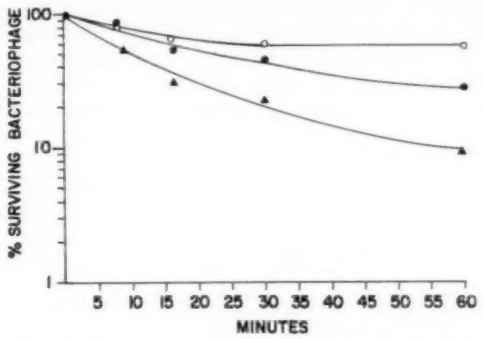

FIG. 5. Rate of inactivation of T2r+ coliphage (10^4 p.f.u. per ml.) by 100 (○), 200 (●), and 400 (▲) microgm. per ml. of lysozyme.

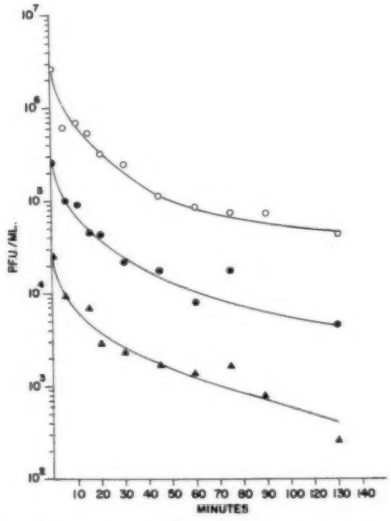

FIG. 6. Rate of inactivation of 2.5×10^4, 2.5×10^5, and 2.5×10^6 p.f.u. per ml. of T2r+ coliphage by lysozyme (500 microgm. per ml.).

Susceptibility of Lysozyme-Treated E. coli to Bacteriophage Infection

The studies described in the preceding paragraphs showed that the degree of bacteriophage inactivation was markedly influenced by variations in condition of incubation of phage with lysozyme prior to mixing with susceptible bacteria. Although these observations strongly suggested a direct effect of lysozyme on bacteriophage particles, an additional action of lysozyme on *E. coli* rendering them less susceptible to lysis was not excluded.

Lysozyme in a final concentration of 1000 microgm. per ml. was added to a 4 hour culture of *E. coli* in trypticase soy broth. After incubation for 30 minutes at 37°C., bacteria cells were sedimented by centrifugation at 2000 r.p.m. and resuspended in broth. A culture of *E. coli* without added lysozyme served as a control. These bacterial suspensions were used immediately for titrations of several preparations of T2 bacteriophage.

No significant difference was observed in the number of p.f.u. formed on plates prepared with lysozyme-pretreated or control *E. coli*. Mahler and Fraser (10) have shown that *E. coli* protoplasts formed by digestion of cell walls with lysozyme can support replication of T3 coliphage.

Residual Lysozyme Activity After Inactivation of Bacteriophage

Complete inactivation of bacteriophage was not achieved with lysozyme in concentrations as great as 500 microgm. per ml. (Fig. 4). As shown by the following experiments, the persistence of active phage particles was not due to a decrease in phage-inactivating activity of the lysozyme solution.

Fifty ml. of a solution of lysozyme in a concentration of 250 microgm. per ml. in phosphate buffer (0.1 M, pH 6.5) was placed in a water bath at 37°C., and at 0, 1, 2, 3, and 4 hours of incubation 0.05 ml. of a suspension of T2 bacteriophage was added to bring the final phage concentration to 10^8 p.f.u. per ml. Aliquots were removed immediately before and after each addition of bacteriophage for determination of p.f.u. and bacteriolytic activity.

There was no significant difference in the degree of inactivation of 5 separate doses of bacteriophage added sequentially to a single solution of lysozyme (Table I). In addition, the bacteriolytic activity of the lysozyme solution did not change as a result of repeated inactivation of bacteriophage.

Additional experiments using minimally detectable concentrations of lysozyme and large doses of bacteriophage were undertaken to determine if lysozyme was adsorbed to phage particles.

TABLE I

*Inactivation of Repeated Doses of T2r+ Bacteriophage by a Single Solution of Lysozyme**

Time of Addition of 10^8 p.f.u. per ml.	Bacteriophage Inactivated After 1 Hour
Hours	Per cent
0	87
1	85
2	76
3	89
4	67

* 250 microgm. per ml. in sodium phosphate buffer (0.1 M, pH 6.5) containing 1 mg. per ml. serum albumin.

Bacteriophage and lysozyme in sodium phosphate buffer (0.1 M, pH 6.5) were mixed to give a final concentration of 0.5 microgm. per ml. of lysozyme and 10^9 p.f.u. per ml. of phage. Aliquots of the mixture were placed at 4° and 37°C. for 1 hour and then centrifuged for 30 minutes at 25,000 r.p.m. in a Spinco ultracentrifuge. Similarly processed controls contained only lysozyme or bacteriophage. After centrifugation, the concentrations of lysozyme and bacteriophage in the supernatant fluid were determined.

No change in concentration of lysozyme could be detected after centrifugation of phage-lysozyme mixtures despite removal of 95 to 99 per cent of active bacteriophage. The lysozyme solutions used in these experiments contained approximately 2.1×10^{13} molecules per ml., assuming that the molecular weight of lysozyme is 14,000. Thus in order to reduce the lysozyme concentration from 0.5 to 0.4 microgm. per ml., 20 per cent or 4×10^{12} molecules would have to be adsorbed to 10^9 bacteriophage particles (4,000 molecules per phage). Although this experiment provides no evidence for adsorption of lysozyme, the adsorption of less than 4,000 molecules of lysozyme per phage particle cannot be excluded.

Dissociation of Phage-Inactivating and Bacteriolytic Activities of Lysozyme

The best known property of lysozyme is its specific enzymatic action on the cell walls of certain bacteria (13). This property of the lysozyme molecule can be separated from its phage-inactivating action as shown in the following experiment with heated lysozyme.

Aliquots of a solution containing 1000 microgm. per ml. lysozyme in sodium phosphate buffer (0.1 M, pH 6.5) were incubated at 4°, 56°, 77°, 88°, and 100°C. for 30 minutes. After incubation, each aliquot was tested for phage-inactivating and bacteriolytic activity.

Lysozyme heated at 56°C. for 30 minutes retained in full its capacity to lyse *M. lysodeikticus* and inactivate purified T2r+ bacteriophage (Table II). In contrast, the bacteriolytic activity of lysozyme heated at 77° or 88°C.

TABLE II

Dissociation of Bacteriolytic and Phage-Inactivating Effects of Lysozyme by Heat

Preparation of Lysozyme*	Lysozyme Activity	
	Bacteriolytic microgm. per ml.	Amount required to inactivate 50% of phage microgm. per ml.
Control		
4°C.	1000	45
Heated		
56°C.	1100	50
77°C.	560	20
88°C.	310	60
100°C.	not done	>300

* Incubated at designated temperature for 30 minutes.

was reduced two or threefold but its phage-inactivating action was not altered. Lysozyme heated at 100°C. for 30 minutes was devoid of activity.

Inhibition of Phage Inactivation by Deoxyribonucleic Acid and Bacterial Endotoxin

The bacteriolytic activity of lysozyme (11) and the bactericidal effect of histone (12) can be blocked by certain acidic polymers. As shown in Table III, inactivation of T2r+ phage by lysozyme was inhibited by deoxyribonucleic acid or *E. coli* endotoxin. Only approximately 40 per cent inactivation of 10^4 p.f.u. per ml. was achieved by 250 microgm. per ml. of lysozyme in the presence of DNA or endotoxin, whereas 80 per cent inactivation was obtained by lysozyme solution alone.

Attempts to Reactivate Lysozyme-Inactivated Bacteriophage

Andrews and Elford (14) found that bacteriophage incompletely neutralized by antiserum could be partially reactivated simply by dilution and subsequent incubation of neutralized virus. Utilizing this technique, it was not possible to reactivate T2r+ inactivated by lysozyme.

A mixture of bacteriophage (10^9 p.f.u. per ml.) and lysozyme (300 microgm. per ml.) in sodium phosphate buffer (0.1 M pH 6.5) was incubated for 1 hour at 37°C. A suspension of bacteriophage (10^9 p.f.u. per ml.) with no added lysozyme served as a control. After incubation, the solutions were rapidly diluted a hundred thousandfold in buffer and placed in a water bath at 37°C. Aliquots were removed from each solution after 0, 1, 2, 3, and 4 hours and diluted to give approximately 125 p.f.u. per plate.

As is apparent from the data presented in Table IV the number of p.f.u. did not increase despite a hundred thousandfold dilution and incubation of the lysozyme-inactivated preparation at 37°C. for 4 hours.

Mandel (15) has reported reactivation of poliomyelitis virus neutralized by antiserum by dilution of the virus-antibody complex in buffers of low pH. However, dilution of lysozyme-inactivated bacteriophage in 0.1 M acetic acid-sodium acetate buffers of pH 3.0, 4.0, 5.0, and 6.0 and incuba-

TABLE III

Effect of DNA and Bacterial Endotoxin on T2r+ Phage Inactivation by Lysozyme

Materials Tested	Bacteriophage* Inactivated Per cent
Lysozyme, 250 microgm. per ml.	80
Lysozyme, 250 microgm. per ml. and DNA, 1250 microgm. per ml.	32
Lysozyme, 250 microgm. per ml., and endotoxin, 1250 microgm. per ml.	47
DNA or endotoxin, 1250 microgm. per ml.	0

* 10^4 p.f.u. per ml.; reaction carried out in sodium phosphate buffer (0.1 M., pH 6.5) with 0.1 per cent albumin; incubation 37°C. 1 hour.

TABLE IV

Effect of Dilution and Incubation on Lysozyme-Inactivated T2r+ Bacteriophage

Hours of Incubation* After Dilution	Bacteriophage Control p.f.u. per plate	Lysozyme-Inactivated Bacteriophage p.f.u. per plate	Bacteriophage Inactivated Per cent
0	125	27	78
1	127	26	80
2	127	29	75
4	120	28	77

* 37°C.

tion at 26°C. for 1 hour did not bring about detectable reactivation. These experiments were complicated by the fact that at pH 3 or 4 considerable inactivation of phage occurred in control suspensions containing no lysozyme. In additional experiments, no detectable reversal of the inactivation process was obtained by treating lysozyme-inactivated phase with trypsin or papain (1 hour, 37°C.). Jerne and Maaloe (8) were able to reactivate completely T4 coliphage previously inactivated by ribonuclease by incubation with trypsin. It is also of interest that Kalmanson and Bronfenbrenner (16) and Hershey (17) found that neutralized coliphage "PC" and P9H phage could be completely reactivated by digestion of the antibody with papain.

Effect of Lysozyme on Unpurified T2r+ Coliphage

Early in the course of these studies it was found that T2r+ bacteriophage present in crude lysates of *E. coli* was relatively insensitive to inactivation by lysozyme. In repeated experiments, less than 30 per cent decrease in p.f.u. of unpurified T2r+ was achieved with lysozyme in a concentration sufficient to inactivate 90 per cent of phage purified by the method of Herriott and Barlow (2). This observation suggested that T2r+ was rendered susceptible to the action of lysozyme during the process of purification. To further investigate this possibility, crude phage lysates were purified and phage susceptibility to inactivation by lysozyme was determined after each step in the procedure.

The method of purification was adopted from Herriott and Barlow (1). *E. coli* was grown on a synthetic glucose-ammonium medium at 37°C. until the bacterial concentration had reached approximately 10^8 cells per ml. Bacteriologically sterile T2r+ was then added to the flask to give a final concentration of 10^6 p.f.u. per ml. The culture was then shaken at 60 r.p.m. on a rotating apparatus at 37°C. for about 18 hours at which time lysis was complete. The crude lysates containing 10^9 to 10^{10} p.f.u. per ml. were stored at 4°C. for 2 or more days prior to purification as outlined below.

Step I. "Celite analytical filter aid" (Johns-Manville) was added to the crude lysate to give a final concentration of 0.25 gm. per 100 ml. The suspension was then filtered through number 3 Whatman paper.

Step II. After filtrate from Step I had reached 4°C., 0.1 M HCl was added slowly with constant stirring until the pH had reached 3.9 to 4.0 as measured with a glass electrode. The acidified suspension was allowed to stand at 4°C. for 30 minutes. No clearly visible precipitate formed in the small amounts of filtrate used in these experiments. Nevertheless, the cold acidified suspension was centrifuged at 2400 × g for 30 minutes in a refrigerated International Centrifuge. The supernate was discarded and the residue was resuspended in about one-tenth the original volume of cold saline. One-tenth molar $NaHCO_3$ was then added to bring the pH to 6.5.

Step III. The phage suspension was then treated with DNase in a concentration of 1 microgm. per ml. in the presence of 0.003 M concentration of $MgSO_4$. Digestion was continued for 2 hours at 26°C

The susceptibility of bacteriophage to inactivation by lysozyme after each step of the purification procedure is shown in Table V. Bacteriophage in crude lysates or in lysates after addition of "Celite" and filtration was totally insusceptible to inactivation by lysozyme. However, after acidification to pH 3.9–4.0, centrifugation, resuspension in saline solution and subsequent neutralization to pH 6.5 (Step II), the bacteriophage was as susceptible to inactivation by lysozyme as highly purified T2r+. It is also apparent from the data presented in Table V that treatment with DNase in the presence of Mg++ did not further alter phage susceptibility to lysozyme.

Undoubtedly the step of acid precipitation, centrifugation, and resuspension of phage in saline results in the elimination of cell debris and products of cell disruption that might interfere with the antiphage action of lysozyme. However, it was not necessary to separate the phage from the crude lysate to render it susceptible to inactivation by lysozyme. The simple procedure of acidification of crude lysates to pH 3.9–4.0 at 4°C. and subsequent raising of the pH to 6.5 was sufficient to change bacteriophage from lysozyme-resistant to lysozyme-sensitive. Phage susceptibility to inactivation by lysozyme was evident by 10 minutes after acidification to pH 4 and only slight additional increase in susceptibility was achieved if pH 4.0 was maintained for 1 hour prior to neutralization. In these experiments acidificatoin did not

TABLE V

Effect of Purification on Susceptibility of T2r+ Bacteriophage to Inactivation by Lysozyme

Steps of Purification Procedure	Bacteriophage Inactivated Per cent
Crude lysate after addition of celite	0
Crude lysate after addition of celite and filtration	0
After acid precipitation, resuspension in saline solution, and neutralization to pH 6.5	83
After addition of 0.003 M Mg++	85
After digestion with DNAse	88

result in loss of infectivity, presumably because the procedure was carried out at 4°C.

Effect of Lysozyme on Other Coliphages

Chemical inactivation of bacteriophages tends to be type specific and has been utilized in their identification and classification. Labaw, Mosley, and Wyckoff (18) found that T2, T4, and T6 were inactivated by small concentrations of formaldehyde but that T1 was not. Putnam, Miller, Palm, and Evans (19) have shown differences in susceptibility of T6 and T7 to inactivation by cationic detergents. Also Gretchman and coworkers (20) found that T1 and T5 were inactivated by several basic proteins, but that other members of the T series were not affected. Thus it was not surprising to find that only certain coliphages were inactivated by lysozyme and that one strain was much more susceptible than the others.

T1, T2, T3, T4, T6, and T7 coliphages present in dilute crude lysates were tested for inactivation resulting from incubation with lysozyme in a final concentration of 500 microgm. per ml. Because acidification markedly increased the susceptibility of T2 to lysozyme, all phages were retested against lysozyme after the lysates were acidified to pH 4.0, allowed to stand for 30 minutes at 4°C. and then brought to pH 6.5 with 0.1 M $NaHCO_3$ solution. No attempt was made to separate the acid-treated phage from the rest of the material in the lysate.

As shown in Table VI, lysozyme occasionally had slight activity against T2 bacteriophage present in crude lysates and was markedly active against acid-treated T2 bacteriophage. None of the other coliphages present in

TABLE VI

Effect of Lysozyme on Other Coliphages

Bacteriophage	Bacteriophage Inactivated Per cent
Crude lysate	
T1	0
T2	0–30
T3	4
T4	0
T6	0
T7	7
Acid-treated lysate*	
T1	0
T2	90
T3	0
T4	13
T6	20
T7	0

* Crude lysate acidified to pH 4.0, held at 4°C. 30 minutes, then neutralized to pH 6.5

TABLE VII

Inactivation of Certain Coliphages by Calf-thymus Histone

Bacteriophage	Bacteriophage Inactivated Per cent
Crude lysate	
T1	94
T2	67
T3	15
T4	97
T6	86
T7	22
Acid-treated lysate*	
T1	43
T2	100
T3	30
T4	91
T6	89
T7	0

* Crude lysate acidified to pH 4.0, held at 4°C. 30 minutes, then neutralized to pH 6.5

crude lysates was inactivated by lysozyme and only slight inactivation of T4 and T6 was demonstrated after acidification.

Effect of Histone on Coliphages

Histone has been shown to have potent bactericidal activity against coliform bacilli and micrococci under certain conditions *in vitro* (12). As shown by the following experiment, calf-thymus histone also has impressive antiphage activity.

Calf-thymus histone was dissolved in 0.01 N HCl, 10 mg. per ml., and was then diluted to appropriate concentration in sodium phosphate buffer (0.1 M, pH 6.5) containing 0.1 per cent serum albumin. Histone in a final concentration of 100 microgm. per ml. was tested against coliphages by the method described in the preceding section.

The spectrum of coliphage-inactivation by histone differed markedly from that of lysozyme. Histone was quite active against T1, T2, T4, and T6 present in crude lysates (Table VII). Acidification did not markedly alter the sensitivity of any of the coliphages to histone.

DISCUSSION

The present studies show that lysozyme, an enzyme that catalyzes the splitting of cell walls of certain bacteria, inactivates purified T2r+ bacteriophage. Several other enzymes with high isoelectric points have also been shown to inactivate bacteriophage. In 1938, Northrop (21) demonstrated that staphylococcal phage was inactivated after incubation with chymotrypsin. However, the protein nitrogen in the bacteriophage suspen-

sion did not change during treatment with chymotrypsin, suggesting that inactivation was not associated with enzymatic digestion of phage protein. Recently, Jerne and Maaloe (8) observed that crystalline ribonuclease in a concentration of 1000 microgm. per ml. inactivated coliphage T4r. This inactivation was probably not enzymatic either, since coliphages do not contain ribonucleic acid, and neutralized phage could be completely reactivated by treatment with trypsin. It is likely that the phage-inactivation described in the present studies does not result from the classical enzymatic action of lysozyme on phage. This assumption was made because heating to 88°C. for 30 minutes decreased the bacteriolytic activity of lysozyme but did not alter its capacity to inactivate phage.

In the absence of evidence for a specific enzymatic action of these substances on bacteriophage it is possible that phage inactivation by lysozyme, chymotrypsin, and ribonuclease is related to the basic nature of these proteins. The finding of phage inactivation by several other basic proteins *without* known enzymatic activity supports this view. Watson *et al.* (22) showed that a basic polypeptide made of lysine could inactivate *Clostridium madisonii* bacteriophage as well as other viruses and bacteria. Inactivation of coliphage T1 by protamine and histone was recently described by Fischer and Brandis (24) and by Gretchman and associates (20) who found that several basic proteins including protamine, clupein and salmine inactivated T1 and T5 but not other members of the T series. In their experiments calf-thymus histone was active only against T1 phage, in contrast to the present studies which show inactivation not only of T1 but also of T2, T4, and T6 by histone.

The mechanism of inactivation of bacteriophages by basic proteins is unknown. Phage inactivation could be explained by a union of positively charged groups on the protein molecule with negatively charged areas on the bacteriophage resulting in phages incapable of attachment to susceptible bacteria. A similar hypothesis has been advanced by Stahmann *et al.* (23) to account for the inactivation of tobacco mosaic virus by a synthetic lysine polypeptide. At high phage concentrations the union of basic protein and phage might result in clumping and apparent decrease in number of viable units of phage. However, a number of observations indicate that clumping does not account for the decrease in plaque forming units after incubation of a low concentration of phage with histone or lysozyme. Fischer and Brandis (24) did not detect clumping or other morphologic changes in electron micrographs of histone inactivated bacteriophage. In the present studies the possibility of clumping seemed remote since the rate of inactivation and the proportion of phage inactivated were not influenced by changing the concentration of phage. If aggregation of infectious par-

ticles accounted for the decrease in plaque count, the rate of inactivation should vary with the concentration of bacteriophage and should be more rapid in concentrated than in dilute suspensions of phage.

Reactivation of T2 phage inactivated by lysozyme could not be demonstrated by several different methods. Nevertheless, it is clear from the studies of Jerne and Maaloe (8) that the reaction between bacteriophages and basic proteins may under certain conditions be reversible. These investigators showed complete reactivation of T4 inactivated by ribonuclease after incubation with trypsin. However, Gretchman and Kaplan (9) were unable to demonstrate by dilution and incubation, reactivation of T1 or T5 inactivated by protamine.

Inactivation of purified T2r+ by lysozyme proceeds rapidly until about 80 per cent of the phage is inactivated but after 20 minutes the rate of inactivation decreases sharply. The change in rate of inactivation and the persistence of a residue of active bacteriophage are not due to a lack of active lysozyme, for if additional phage is added to a mixture of phage and lysozyme after the rate of inactivation has decreased the second dose is inactivated as rapidly as the first. Gretchman and Kaplan (9) recorded similar observations on the kinetics of inactivation of T1 and T5 by protamine, and showed that the phages resisting inactivation were not protamine-resistant mutants. It is likely that the slowing of rate of inactivation and the persistence of a residue of active bacteriophage is related to lack of uniform susceptibility of the bacteriophage population to inactivation by lysozyme. This hypothesis has been proposed as an explanation of persistence of a small percentage of active bacteriophage in mixtures of phage and excess antibody. Another possible explanation is similar to that advanced by Gard for the inactivation of poliovirus for formaldehyde; that is, that *during the course* of inactivation some of the virus particles become less susceptible to the action of the inactivating substance (25).

It is of considerable interest that T2r+ bacteriophage present in crude lysates was rendered susceptible to inactivation by lysozyme by the simple procedure of acidification to pH 4.0 and subsequent neutralization. The mechanism of this effect is unknown. It is possible that acidification results in damage to the phage particle without loss of infectivity and that the damaged phage is more susceptible to inactivation by lysozyme. Another explanation is that acidification results in dissociation of phage from some product possibly of bacterial origin that blocks the attachment of lysozyme. Hershey *et al.* (6) have shown that crude coliphage PC is probably combined with bacterial nucleoproteins, and Putnam and coworkers (26) have demonstrated that desoxyribonucleic acid accompanies T6r+ throughout the course of purification by several methods and that the amount of DNA is decreased by acidification.

SUMMARY

Crystalline egg-white lysozyme inactivates the infectivity of *purified* T2r+ bacteriophage by direct action on phage particles. Inactivation is influenced by concentration of lysozyme, time and temperature of incubation of phage-lysozyme mixtures, and molarity and pH of the suspending medium. Heating lysozyme decreases its enzymatic lysis of *Micrococcus lysodeikticus* but does not impair its capacity to neutralize bacteriophage. Deoxyribonucleic acid or *E. coli* endotoxin inhibit phage inactivation by lysozyme.

Non-purified T2r+ in crude lysates is resistant to inactivation by lysozyme, but acidification of crude lysates to pH 4.0 changes the phage from lysozyme-resistant to lysozyme-sensitive.

Members of the T series of coliphage other than T2 are not inactivated by lysozyme.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY IN ASSOCIATION WITH THE ALPHA OMEGA ALPHA FRATERNITY

May 23, 1960

HORMONAL AND IONIC INFLUENCES ON UTERINE ACTIVITY

ELWIN BERGER

Uterine activity is known to be regulated by estrogen and progesterone. Furthermore, the effectiveness of oxytocin as a stimulant is influenced by the hormonal state of the uterine muscle. Recently it has been suggested that the action of oxytocin is also affected by the extracellular calcium and potassium concentrations. The present experiments were done to investigate further these findings.

Ovariectomized white rats were divided into three groups: 1—estrogen-treated, 2—progesterone-treated, and 3—untreated controls. At the end of the treatment period the animals were sacrificed and their uterine horns removed. The uterine segments were placed in an organ bath containing Krebs solution at 37°C. and their contractions were recorded isometrically.

The uterine muscles in all three groups exhibited spontaneous rhythmical activity. The estrogen-treated group, however, was the most sensitive to the stimulatory action of oxytocin. All three groups also responded similarly to increases in the concentrations of extracellular potassium up to ten times normal, and responses to oxytocin were unchanged except for a slightly increased response in the progesterone-treated group.

The absence of external calcium completely inhibited the spontaneous activity of all three groups in ten to fifteen minutes. Under these conditions the response of the muscles to oxytocin was greatly diminished although the reaction of the estrogen-treated group remained comparatively greater than that of the other two groups. When the extracellular calcium was reduced to one-tenth of the normal concentration, the spontaneous activity in the estrogen-treated muscles was completely abolished whereas the progesterone-treated muscles continued to contract. Also the response to oxytocin under these conditions was slightly greater than that seen in calcium-free solutions although not as great as that in normal calcium solutions. The results seem to indicate that oxytocin is dependent on both calcium and estrogen for its stimulatory action on the uterus.

MYOCARDIAL METABOLISM DURING CORONARY PERFUSION WITH GASEOUS OXYGEN

DARRYL CARTER

In the recent past it has been demonstrated here that the isolated canine heart will continue to beat for periods up to eight hours when the coronary circulation is perfused solely with oxygen in the gaseous state. Similar preparations without gaseous oxygen perfusion or with perfusion of nitrogen arrest in an average of 18 minutes. The present studies were performed on 30 isolated hearts which were quickly removed from the chest and subjected to one of three experiments. In the first group the heart was rapidly frozen in dry ice and the glycogen and lactic acid contents were determined. In the second group the heart was

placed in a humidified plastic chamber at 37°C. and the coronary arteries were cannulated and perfused with gaseous oxygen. The heart beat continued for a period of several hours after which glycogen and lactate values were determined. The hearts in the third and control group were placed in the plastic chamber without perfusion of the coronary circulation and allowed to remain suspended for periods up to seven hours. The results were as follows:

Average Values of Myocardial Glycogen and Lactate

	No.	Glycogen (mg. per 100 gm.)	Lactate (μm per 100 gm.)
Normal	8	533	58
O_2 Perfusion	11	336	26
Controls without O_2 Perfusion	11	56	225

These data indicate that the myocardium is capable of utilizing oxygen when perfused in the gaseous state. Glycogen is metabolized aerobically and lactate does not accumulate in the tissues.

STUDIES WITH HEPARIN AND PROTAMINE IN EXTRACORPOREAL CIRCULATION

Michael E. Jabaley, Paul Geary, Jr., David L. Glancy, Terry W. Taylor, Ronald K. Tompkins, Frank C. Spencer and Henry T. Bahnson

In light of the variation which exists concerning heparin dosage and neutralization, the following studies were performed on 66 patients undergoing open heart surgery, all of whom received 3 mg. heparin per kg. initially. These studies employed prothrombin times, by the method of Quick, and protamine titrations, by a modification of the method of Leroy, *et al.*

Prothrombin times on patient's plasma to which heparin had been added *in vitro* and *in vivo* demonstrated a wide range of heparin activity. The significance of this variation is unclear and there was no correlation with the clinical course, but it emphasizes that one cannot say with certainty what range of heparin activity is necessary to insure adequate anticoagulation during perfusion.

The effect of prolonged perfusion on plasma heparin levels was studied in a group of patients pumped one hour or longer who received supplementary doses of heparin at 30 minute intervals. Decreases in prothrombin time and the response to extra heparin was demonstrated. Current practice is to give 1 mg. heparin per kg. after 30 minutes on bypass and 0.5 mg. per kg. after each succeeding 30 minutes. No improvement in 24 hour chest drainage was noted in these patients, but no untoward effects were seen from the extra heparin and it was possible to maintain the prothrombin times at a relatively constant level.

In 28 patients with known blood volumes receiving specific heparin doses, calculations of plasma heparin concentration were made. These were compared with heparin concentrations derived from protamine titrations of these same patient's plasma. Calculated levels were from 1.65 to 4.40 times greater than titratable levels, indicating that one cannot perform such a procedure to estimate the protamine dose necessary to neutralize heparin after perfusion. This was found to be true in clinical practice also.

Protamine and polybrene were compared regarding their effect on 24 hour chest drainage in patients perfused one hour or longer and no significant difference was noted. Polybrene is currently used because of its more rapid action.

STUDIES ON THE PATHOGENESIS OF STAPHYLOCOCCAL INFECTIONS

CHARLES R. CONTI, JR.

During the course of studies on the influence of non-specific inflammation upon infectivity of staphylococci, the effects of endotoxin derived from gram negative bacteria, were examined. Endotoxin can produce leukopenia and interfere with leukocyte diapedesis and motility in rabbits, and it also sensitizes the blood vessels to the vasoconstrictor effects of adrenalin.

An intradermal dose of 10^8 or 10^7 pathogenic staphylococci produces small furuncles in rabbit skin while a dose of 10^6 or 10^5 organisms produces no lesions at all. Ten micrograms of intracutaneous endotoxin produced a pale area but no gross inflammation. When the pathogenic staphylococci in a dose as low as 10^5 organisms were injected into endotoxin prepared skin within two hours, striking lesions characterized by extensive necrosis and hemorrhage appeared. Ten micrograms of intravenous endotoxin also influenced the staphylococcus skin injected areas, leading to development of necrosis and hemorrhage. Nonpathogenic staphylococci failed to induce lesions such as those produced with pathogenic staphylococcus. Dermonecrotic alpha hemolysin and heat killed staphylococcus were not potentiated by endotoxin. Cell free exudate, from rabbits with staphylococcal peritonitis, plus alpha toxin, failed to reproduce the reaction observed with viable pathogenic bacteria.

Sympatholytic agents failed to inhibit the reaction of endotoxin upon staphylococcus infection, even though these drugs are known to prevent the vasoconstricting effects of endotoxin. Cortisone increased the effect of endotoxin upon staphylococcus infection.

Leucopenia (which occurs abruptly after injection of endotoxin and persists for 2 to 3 hours) and decrease of diapedesis and migration of leukocytes, by endotoxin, most likely is the mechanism whereby the host's defense against the staphylococcus is impaired. This also suggested that the increased infectivity of staphylococci, produced by endotoxin, leads to greater bacterial multiplication and production of sufficient hemolysis locally, to lead to dermonecrosis.

A SMOOTH MUSCLE ASSAY IN THE STUDY OF HYPERSENSITIVITY

HARRY G. RANDALL AND HAROLD C. NEU

The use of an isometric Schulz-Dale apparatus in studies of experimental anaphylaxis permits highly reproducible estimates of smooth muscle contraction. The dose response relationships of ovalbumin, human gamma globulin, and pneumococcal polysaccharide SIII were characterized, and the differences among these systems discussed. Data were presented to show that normal gamma globulin may compete with immune gamma globulin for antigen receptor sites in the guinea pig small intestine. *In vitro* sensitization with antigen was shown not to be a unique property of the globulin system since it was possible to fix both protein and polysaccharide antigens to guinea pig small intestine. The utility of this model for study of tissue damage as a result of immune reactions was indicated.

RELATIONSHIP BETWEEN POTASSIUM BINDING AND OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA

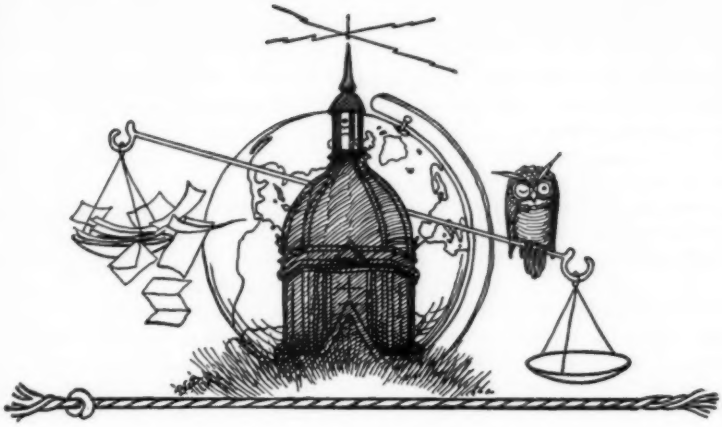
ROBERT L. SCOTT

The importance of mitochondria as the main locus of carbohydrate metabolism within the cell is well recognized. These organelles are the site of the actual trapping of carbohyrate energy in a usable form by taking up inorganic phosphate and incorporating it into adenosine-di-phosphate to convert it to the energy rich compound adenosine-tri-phosphate

Mitochondria are known to bind potassium specifically and tightly in such a way that it cannot be removed by repeated washings of the mitochondria in isotonic sodium chloride or isotonic sucrose solutions. However, this bound potassium will exchange with ambient potassium if mitochondria are incubated in a solution containing free, radioactively-labeled potassium.

In this study organic mercurials and inorganic mercuric salts were found to stimulate the rate of exchange, destroy the binding capacity for potassium and destroy the capacity for oxidative phosphorylation. These three events could be brought about in various combinations depending on how the mercuric ion was presented to the mitochondria. However, under no circumstances was it possible to destroy the potassium binding capacity of the mitochondria while maintaining their capacity to carry out oxidative phosphorylation.

It was also observed that potassium discharge in salt free solutions resulted in a significant rise in pH of the solution and again simultaneous loss of the capacity to carry out oxidative phosphorylation occurred.



BOOK REVIEWS

A Manual of Tropical Medicine, 3rd edition. By GEORGE W. HUNTER, III, WILLIAM W. FRYE AND J. CLYDE SWARTZWELDER. 892 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

The world has become small, transoceanic distances negligible, and the economics and politics of the underdeveloped and overpopulated countries are rapidly changing. "Western" or allopathic medicine is replacing indigenous, herb, or Ayuravedic medicine, and is being brought for the first time to vast numbers of sick persons the world over. As part of this, since World War II, Americans official and unofficial, "quiet" and "ugly," have spread themselves all over the world. Most of them return home after relatively brief and limited exposures to "tropical" or exotic diseases.

The continuing success of "manuals on the diseases of warm climates" since Manson popularized the subject with his first edition of "Tropical Diseases" some 60 years ago attests to the real demand for this somewhat artificial collection of subjects. Many diseases such as epidemic typhus, trichinosis, and infection with fish tapeworm do better in cold climates, but are classical "tropical diseases." The material covered in a standard text such as this excellent one, now in its third edition, thus includes a unique mixture of insect transmitted diseases, vitamin deficiencies, helminths and protozoa. If Americans are to contribute to a partial defaunation of "this wormy world," they need to know a great deal more about just what and where these diseases are. This Manual of Tropical Medicine is of great assistance in this. It is edited by three men who have themselves contributed greatly to the subject. It is interesting that all three were trained originally as parasitologists, and all have worked with human disease. Numerous experts handle individual chapters, thus making this the most concise and perhaps most authoritative text available today. Only one word of serious complaint to the publisher. The enquiring reader who wants to know the basis of a particular statement can find no specific reference to turn to. If he wants to know the evidence for the nerve cell damage which produces megacolon in Chagas' disease, or the rationale behind antimony treatment of schistosomiasis, or the evidence, if any, for a viral etiology of tropical eosinophilia, he must start the search by himself. The inclusion of a list of various texts and journals in the front is no substitute for this need.

This manual was put together during World War II, as a result of a course for Army Medical officers about to go overseas. May it continue to answer needs far beyond those of the temporary visitor.

FREDERIK B. BANG

Xylocaine. The Pharmacological Basis of Its Clinical Use. By STEN WIEDLING. 146 pp., $4.00. Originally published in Swedish by Almqvist & Wiksell, Stockholm, Sweden, 1960. Current English edition available in the U. S. upon request to Astra Pharmaceutical Products, Inc., 7 Neponset St., Worcester 6, Mass.

This small, concise book, originally written in Swedish, is appropriately named. Although it treats all aspects of the pharmacology of Xylocaine, particular emphasis has been placed on considerations closely relating to clinical applications. A number of tables and figures illustrate nicely the similarities and differences between this drug and procaine in causing nerve block. Surface anesthesia is less fully discusssed. One might wish for more comparisons of Xylocaine with other useful local anesthestics, but an attempt to provide these would have undoubtedly presented many difficulties and caused the book to lose much of its clarity.

The author is part of the research staff of the Swedish pharmaceutical firm which manufactures Xylocaine. However, if the book is to be regarded as advertising literature, it sets a standard which deserves imitation. Evidence is evaluated in a critical way, and no extravagant claims are made. Practically every statement is documented; the bibliography contains over 300 references.

This book should be of interest to all clinicians who use Xylocaine.

E. W. MAYNERT

Cosmetic Surgery. Principles and Practice. By SAMUEL FOMON. 651 pp., $27.50. J. B. Lippincott Co., Philadelphia, Pa., 1960.

This is a large and copiously illustrated treatment of the general field of cosmetic surgery with a 230 page introductory consideration of general principles used in the entire field of plastic surgery. An extremely detailed and interesting section of nearly 200 pages is devoted to rhinoplastic procedures, and is, I believe, the most important section of the book. The remaining portion of the book is devoted to cosmetic care of deformities of the rest of the body. There is very little material related to the extremities, but the coverage of the eye, ear, face and breast is in some detail.

The chief value of this book is the presentation of Dr. Fomon's ideas concerning surgery of the nose. It is in this area that the photographic illustrations are best. The book is well indexed and has several pages of references at the end of each section.

It would be primarily recommended as a library edition for those persons more than casually interested in the general field of plastic surgery.

ROBERT W. JOHNSON, III

Principles of Public Health Administration, 3rd edition. By JOHN J. HANLON. 714 pp., $10.50. The C. V. Mosby Co., St. Louis, Mo., 1960.

In the third edition of this excellent text on public health administration, Dr. Hanlon did not make any extensive revisions, but brought the book up to date in several aspects. It is fortunate that the book has not been radically revised, for in its present format it is of great utility to the physician who wishes to learn something of the complex problems of health and medical administration.

Some of the principle strengths of this book that carry through from the earlier edi-

tions are the excellent chapters on organizational problems, personnel factors, and fiscal management. Dr. Hanlon has gone back to the original work of Urwick, Dimock, etc., and applied their principles of management to public health activities. Throughout this volume Dr. Hanlon sticks closely to his title and does not merely describe public health but deals extensively with the administrative problems involved in the many facets of activity that make up public health practice today. Dr. Hanlon has succeeded in covering all of the important aspects of the classical public health programs, as well as the recent innovations. Although the coverage is somewhat thin in areas such as laboratory service and mental health, this is not a serious defect.

One of the principal changes that Dr. Hanlon instituted in this edition is the addition of a historical and philosophical chapter on "The Past as Prologue". This thoughtful exposition and phrophecy is most worthwhile. Dr. Hanlon has also added a complete new chapter on the addictive diseases reflecting the interests of health departments in these problems. Poison control and hazards from sheet plastic have been added to the chapter on accidents, and the chapters on both communicable diseases and chronic diseases have been expanded to some extent as far as topics are concerned.

The section on international public health from the third edition has been cut down somewhat and placed in two other chapters. This is rather unfortunate as Dr. Hanlon is a recognized authority in the field of international health, and this subject is attaining increasing importance for public health workers today. Although Dr. Hanlon's material on international health in the third edition is excellent, it is too brief.

One of the new areas that Dr. Hanlon included in his revision is the question of population. It is interesting to note that Dr. Hanlon does not refer to this as a population "problem" and makes no mention of family planning or population control in relation to health programs. With the emphasis on these activities in Health Departments of many countries of the world today, this is a rather serious omission.

In conclusion, I would strongly recommend this book to anyone who expects to engage in or have any contact with administration in the field of public health.

TIMOTHY D. BAKER

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THE USE OF COLISTIN IN THE TREATMENT OF INFECTIONS OF THE URINARY TRACT[1]

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Despite the tremendous advances in antibacterial chemotherapy during the past 20 years, treatment of chronic and complicated infections of the urinary tract has been generally unsatisfactory. Patients who have underlying structural defects of the excretory system and who have been subjected to instrumentation and prior antimicrobial therapy are particularly prone to invasion by antibiotic-resistant pathogens. Bacteria of the genera Proteus, Klebsiella (Aerobacter), and Pseudomonas are isolated from the majority of such patients. The refractoriness of these organisms to most available antimicrobials is well known and has resulted in a continued quest for agents to which they might be sensitive.

Colistin is a new antibiotic isolated by Koyama and associates (1) from the microorganism *Aerobacillus colistinus*. It is active primarily against Gram-negative bacteria, its antimicrobial spectrum resembling that of polymyxin B (2). Preliminary studies indicate that colistin is bactericidal *in vitro* against many antibiotic-resistant strains of Pseudomonas and Klebsiella. Since these pathogens are frequently associated with refractory infections of the urinary tract, evaluation of colistin in bacilluria due to Pseudomonas and Klebsiella as well as other Gram-negative species seemed warranted.

The studies to be reported consist of the results of *in vitro* sensitivity tests of 181 strains of Gram-negative bacilli against colistin and 5 other antibiotics. The present report also describes our experience with colistin in the treatment of 12 patients who had 13 episodes of urinary tract infection caused by antibiotic-resistant bacteria.

MATERIALS AND METHODS

Organisms: The bacteria tested consisted of 181 urinary pathogens, and only strains present in urine in significant numbers (more than 100,000 colonies per ml.) were

[1] This study was performed under a contract with the Army Chemical Corps, Fort Detrick, Md., and was supported in part by Training Grant E-5 of the U.S.P.H.S. Sodium colistinmethanesulfonate was supplied by the Warner-Lambert Research Institute, Morris Plains, New Jersey.

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studied (3). Nineteen isolates consisted of *Escherichia coli*, 19 of *Klebsiella aerogenes*, 15 of *Pseudomonas aeruginosa* and 4 of Paracolon species. There were 124 strains of Proteus, 91 of which consisted of *P. mirabilis*. The large number of Proteus strains was tested as part of another study (4) of the sensitivity of these organisms to antimicrobials and does not reflect their incidence as pathogens of the urinary tract.

In Vitro Studies: The sensitivity of all isolates was tested against streptomycin, tetracycline, chloramphenicol, polymyxin B, kanamycin and colistin. A tube-dilution method measuring both bacteriostatic and bactericidal end-points was employed. This method has been described in detail in previous publications from this laboratory (5).

Clinical Studies: Thirteen bouts of bacteriuria occurring in 12 patients were treated with colistin. All patients had had previous episodes of urinary tract infection, had been subjected to some form of instrumentation of the urinary tract, and had received multiple courses of antibiotics. In most instances the organisms were resistant to all agents except colistin and polymyxin B. Pertinent clinical data are summarized in Table I.

Sodium colistinmethanesulfonate (Coly-mycin M)[4] was employed in dosage of 35,000 to 90,000 units per kg. per day. This is equivalent to 1,750 to 4,500 micrograms per kg. per day of colistin base. The total daily dose was given by the intramuscular route in four divided doses. The organism isolated from each patient was inhibited by 5 or less microgm. per ml. of colistin, a level of drug readily attained in the urine of patients receiving 35,000 units per kg. per day.

Quantitative urine cultures were performed at frequent intervals to evaluate response to therapy. The duration and results of therapy are summarized in Table II.

RESULTS

In Vitro Sensitivity Studies

A. *E. coli* (Fig. 1). Colistin in a concentration of 10 microgm. per ml. effectively inhibited growth of 18 of 19 strains of *E. coli*. Fifteen of 19 strains were also killed by 10 microgm. per ml. of colistin, a concentration of drug easily achieved in the urine when 40,000 units per kg. are administered parenterally. At these low concentrations colistin enjoyed a distinct advantage over polymyxin B and kanamycin. At high concentration, the three drugs were equally active. Tetracycline, chloramphenicol and streptomycin displayed good antibacterial activity against about 50 per cent of strains but only the last-mentioned was bactericidal.

B. *Klebsiella* (Fig. 2). Colistin in a concentration of 10 microgm. per ml. was bacteriostatic for 18 of 19 strains of Klebsiella and in a concentration of 20 microgm. per ml. inhibited all 19 strains. Bactericidal levels of colistin were only slightly higher than bacteriostatic levels. Kanamycin was almost as effective as colistin against the Klebsiella isolates. However, considerably higher concentrations of polymyxin B were required to achieve antibacterial activity equivalent to that observed with colistin.

C. *Pseudomonas* (Fig. 3). Colistin in a concentration of 5 microgm. per

[4] Trademark of Warner-Lambert Research Institute for sodium colistinmethanesulfonate.

TABLE I

Clinical Data on 12 Patients with Urinary Tract Infection Treated with Colistin

Case	Age	Sex	History	Renal Function	Previous Antibiotic Therapy	Organism
1	68	M	Right nephrectomy, 1953. Prostatectomy, 1958. Bacilluria and fever one year before admission.	SUN:27 mg.%	+	Klebsiella
2	4	F	Congenital vesical neck obstruction. Left pyelonephritis.	N	+	Paracolon
3	8	M	Bilateral ureteral strictures. Stricture bladder neck. Left nephrectomy. History right nephrostomy.	SUN:N PSP:20% (2 hrs.)	+	Pseudomonas
4	54	F	Multiple sclerosis. Cord bladder. Diabetes mellitus.	N	+	Klebsiella
5	66	M	Post-prostatectomy.	N	+	Pseudomonas
6	14	M	Cystinosis. Multiple calculi. Right nephrectomy. History left nephrostomy.	N	+	Pseudomonas Proteus rettgeri
7	46	F	Diabetes mellitus. Left nephrectomy for pyelonephritis. Acute right pyelonephritis.	SUN:41 mg.%	+	Klebsiella
8	63	M	Chronic prostatitis.	N	+	E. coli Klebsiella
9	41	F	Diabetic neuropathy. Cord bladder.	SUN:26 mg.% PSP:40% (2 hrs.)	+	E. coli Pseudomonas
10	74	M	Post-prostatectomy. Epidydimoorchitis and seminal vesiculitis.	SUN:28 mg.%	+	Klebsiella
11	34	F	History nephrectomy for tumor. Recurrent pyelonephritis.	N	+	Pseudomonas
12	9	M	Congenital vesical neck obstruction. History left nephrectomy. Right ureteropyelocaliectasis.	SUN:31 mg.%	+	Pseudomonas

+ = positive.
N = normal.

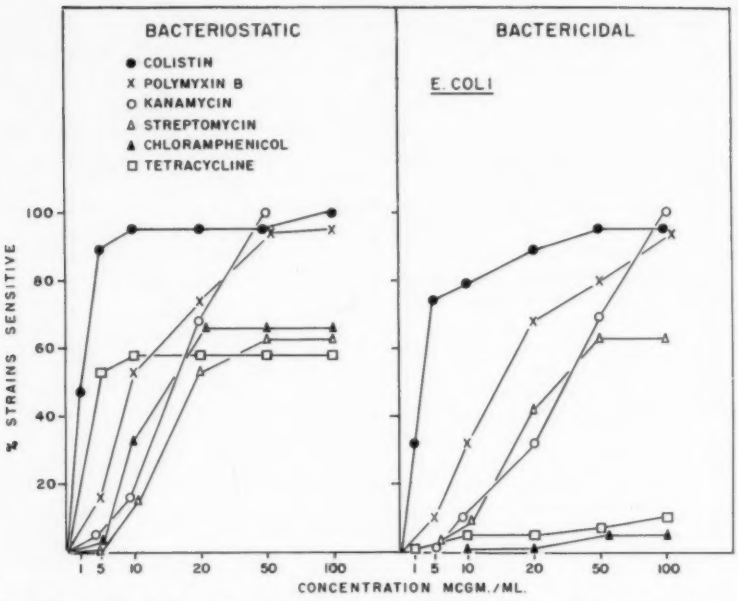

FIG. 1 Bacteriostatic and bactericidal activity of 6 antibiotics against E. coli.

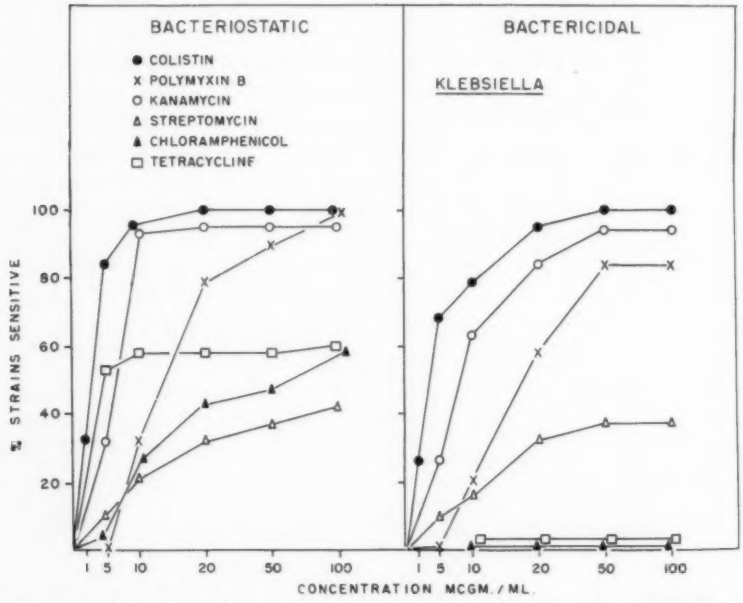

FIG. 2 Bacteriostatic and bactericidal activity of 6 antibiotics against Klebsiella.

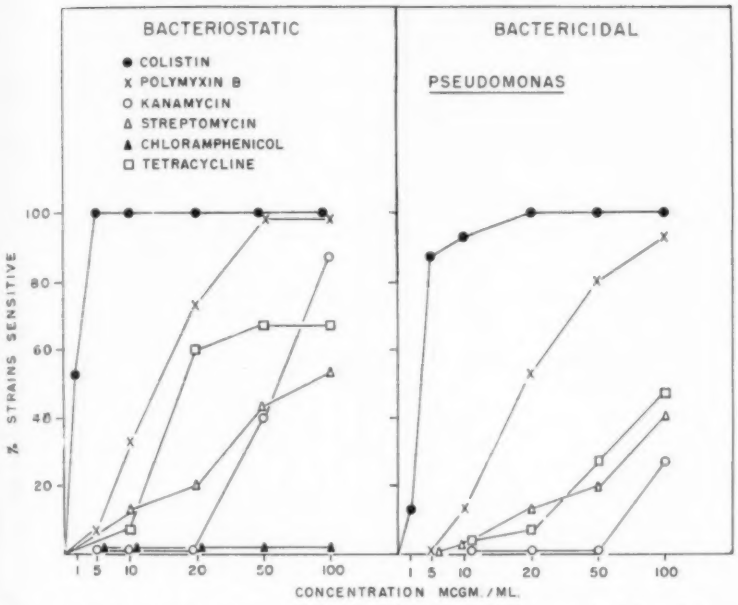

FIG. 3 Bacteriostatic and bactericidal activity of 6 antibiotics against Pseudomonas.

ml. inhibited all 15 strains of Pseudomonas and exerted a bactericidal effect against 13. *In vitro*, it was by far the most potent antimicrobial against these organisms, and only polymyxin B approximated its antibacterial activity. The other drugs tested lagged far behind although relatively good bacteriostasis was achieved with tetracycline.

D. *Paracolon*. Only 4 strains were tested. One was killed by 5 microgm. per ml. of colistin, and the other three were resistant to 100 microgm. per ml.

E. *Proteus*. Only 2 of 91 strains of *P. mirabilis* were sensitive to colistin, and none of 33 strains of *P. morgagni*, *P. vulgaris* or *P. rettgeri* was inhibited by this drug. Polymyxin B was also ineffective against Proteus.

Results of Therapy

All of the patients treated with colistin had chronic and complicated urinary tract infections. As is often the case in patients of this type, the microorganisms isolated from the urine were resistant to most of the commonly used antibiotics. The results of therapy are summarized in Table II.

Colistin was effective in eliminating colistin-sensitive bacteria from the urine of 9 of the 12 patients (Cases 1 to 9). Bacteriologic response usually occurred 24 to 48 hours after beginning antibiotic therapy. Cases 6 and 8

TABLE II

Results of Therapy with Colistin

Case	Organism(s)	No. Colonies	Dose Colistin	Duration of Rx.	Result	Organism After Rx.
1	Klebsiella	3×10^4	2,000,000 u./d. 35,000 u./kg.	10 days	Klebsiella disappeared day 1; E. coli appeared day 6; E. Coli (10^7) present 3 months after Rx. Symptomatic improvement.	E. Coli
2	Paracolon	10^8	1,000,000 u./d. 50,000 u./kg.	7 days	Paracolon disappeared within 24 hours; marked symptomatic improvement.	Strep. fecalis
3	Pseudomonas	10^6	1,500,000 u./d. 60,000 u./kg.	10 days	Urine sterile 48 hours after onset of Rx. Improvement in renal function.	None
4a	Klebsiella	$>10^5$	2,400,000 u./d. 55,000 u./kg.	10 days	Urine sterile within 24 hours. Excellent symptomatic response.	Strep. fecalis
4b	Klebsiella	$>10^5$	2,400,000 u./d. 55,000 u./kg.	10 days	Excellent symptomatic response.	Strep. fecalis Paracolon
5	Pseudomonas	$>10^6$	3,000,000 u./d. 60,000 u./kg.	4 days	Organism disappeared within 24 hours. Therapy discontinued because of emergency surgery.	Staph. aureus
6	Pseudomonas	$>10^6$	2,000,000 u./d.	10 days	Pseudomonas disappeared within 24 hours.	P. rettgeri

6	Pseudomonas Proteus rettgeri	$>10^6$	2,000,000 u./d. 50,000 u./kg.	10 days	Pseudomonas disappeared within 24 hours. P. rettgeri persisted.	P. rettgeri
7	Klebsiella	$>10^6$	3,000,000 u./d. 60,000 u./kg.	7 days	Urine became sterile. Recurrent bacilluria one week after Rx. stopped.	Paracolon Proteus
8	Klebsiella E. coli	$>10^6$ $>10^6$	2,400,000 u./d. 40,000 u./kg.	10 days	E. coli disappeared in 48 hours. Klebsiella decreased transiently to 10^4 org. Symptomatic improvement.	Klebsiella
9	E. coli Pseudomonas	$>10^6$ $>10^6$	2,500,000 u./d. 50,000 u./kg.	7 days	Both organisms disappeared within 24 hours; replaced by P. mirabilis and beta-enterococcus.	P. mirabilis Beta-enterococcus
10	Klebsiella	10^6	4,000,000 u./d. 75,000 u./kg.	10 days	No response. Probable collection of pus in testis or seminal vesicles.	Klebsiella
11	Pseudomonas	10^5	3,000,000 u./d. 75,000 u./kg.	14 days	No. of organisms decreased to low of 10^4 but never disappeared.	Pseudomonas
12	Pseudomonas	10^9	3,000,000 u./d. 90,000 u./kg.	16 days	No. of organisms fell to 5000 but urine never cleared.	Pseudomonas

illustrate the selective antimicrobial action of colistin. In both patients two species of bacteria, one colistin-sensitive and the other colistin-resistant, were cultured from the urine prior to onset of therapy, and in each instance only the sensitive organism was effectively suppressed. It is not surprising in this group of patients that only one (Case 3) remained free of significant bacilluria for as long as 3 months after therapy. After elimination of colistin-sensitive bacteria, the other 8 patients promptly developed significant bacilluria with other organisms.

Cases 1 to 5 had symptoms related to acute cystitis or pyelonephritis at the time therapy with colistin was started. Considerable clinical improvement occurred in these patients coincident with elimination of colistin-sensitive bacteria from the urine. For example, a female (Case 4, Fig. 4) with multiple sclerosis, diabetes and vesical paralysis was admitted with acute Klebsiella pyelonephritis on two occasions. Each time the organism, which was resistant to all other agents, responded to colistin and marked clinical improvement accompanied the disappearance of Klebsiella from the urine. While bacilluria with Streptococcus fecalis ensued following each course of therapy, the patient remained free of symptoms as long as Klebsiella was kept out of the urine.

In three patients (Cases 10, 11, 12) colistin failed to affect the urinary flora other than to produce transient diminution in the degree of bacilluria. Despite the facts that the organisms were quite sensitive to colistin *in vitro* and that these three patients received relatively large doses of

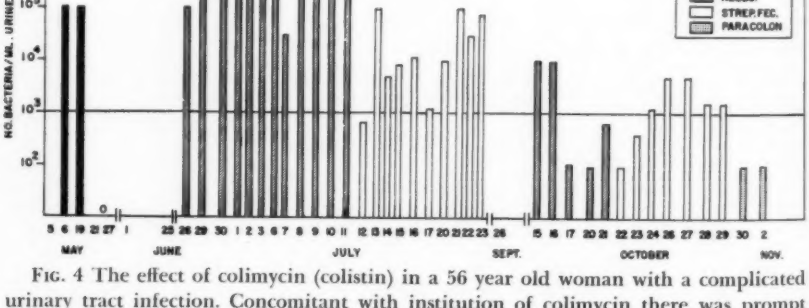

FIG. 4 The effect of colimycin (colistin) in a 56 year old woman with a complicated urinary tract infection. Concomitant with institution of colimycin there was prompt disappearance of Klebsiella from the urine associated with marked symptomatic improvement.

colistin, treatment failed. In one patient (Case 10), this is readily understandable since purulent foci were presumed to exist in the testis and seminal vesicles. The reasons for failure of therapy in the other patients are obscure.

In the dosage employed, colistin was not toxic and the intramuscular injections were not excessively painful. Depression of leukocyte counts did not occur, and no significant abnormalities in other laboratory data were noted.

DISCUSSION

Single bacterial species are cultured from the urine in the majority of patients with acute uncomplicated infection of the urinary tract. In contrast, the urine of patients with chronic and complicated urinary tract infection often yields multiple bacterial species with quite dissimilar antibiotic sensitivities (2). In such instances, evaluation of the effectiveness of an antimicrobial drug must be based on eradication of sensitive bacteria from the urine and not on complete bacteriologic control. Utilizing this approach, the present study shows that colistinmethanesulfonate is capable of eliminating colistin-sensitive Pseudomonas, Klebsiella or Paracolon from the urine of the majority of patients with chronic and complicated infection of the urinary tract. Apparent bacteriologic control occurred in only one of 12 patients. In the others, several organisms resistant to colistin appeared during or shortly after treatment. The close temporal sequence in which these organisms were cultured makes it doubtful that reinfection was their means of entry into the urinary passages. It is more likely that these bacteria were already present in the urine or tissues and made their appearance in large numbers when their predominating rivals were eliminated by the antimicrobial. Whether elimination of colistin-sensitive organisms in favor of bacteria resistant to the drug has a salutary effect on the course of the infection is unknown.

In the absence of more effective agents, however, colistin deserves continued trial in the treatment of infections with certain Gram-negative bacilli. Its activity against strains of Pseudomonas and Klebsiella that are resistant to other antibiotics is encouraging despite the demonstration that therapeutic response does not uniformly parallel the results of *in vitro* sensitivity tests. Fortunately, no untoward effects have been associated with the use of colistin, differentiating it from polymyxin B, an antibiotic with a similar antimicrobial spectrum which is considerably more toxic.

SUMMARY

The sensitivities of 181 strains of Gram-negative bacteria from patients with urinary tract infections were tested against a new antimicrobial,

colistin, as well as polymyxin B, kanamycin, streptomycin, chloramphenicol, and tetracycline. Colistin showed greater bacteriostatic and bactericidal activity against E. coli, Klebsiella and Pseudomonas than any other agent but was ineffective against Proteus species.

The intramuscular preparation of colistin (colistinmethanesulfonate) was employed in 13 bouts of urinary tract infection in 12 patients, all of whom had complicated urinary tract disease which had failed to respond to previous courses of chemotherapy. Sterilization of the urine was achieved in only one patient. However, colistin-sensitive bacteria were eliminated from the urine of 9 of the 12 patients. In 3 patients colistin-sensitive organisms were not eliminated although there was a decrease in the degree of bacteruria during colistin therapy.

Miss Mary C. Rose and Miss Phyllis Weiss rendered valuable technical assistance.

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INHIBITORY EFFECTS OF PASSIVE ANTIBODY ON VIRULENT POLIOVIRUS EXCRETION AND ON IMMUNE RESPONSE IN CHIMPANZEES[1]

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Experimental studies on immune mechanisms in poliomyelitis are of renewed interest at the present time. They not only may improve our understanding of natural infections, but may help also to clarify the consequences of large scale experimental infections of human populations with attenuated viral strains. It is well known that attenuated viruses are capable of inducing infection and immunity in human hosts after simple feeding, under proper conditions. The extent of excretion, spread in open populations, and degree of enhancement of virulence of the progeny of "live virus vaccines" needs further elucidation. Considerable interest attaches to the role of immune mechanisms, and especially humoral antibody, in limiting the spread of virus from individuals infected with natural or attenuated viruses by the oral route.

Although the role of humoral antibody in preventing paralytic poliomyelitis was predicted on the basis of experimental passive immunization (1, 2, 3), and is now generally accepted in relation to immunization of human beings (4), serious doubts are still expressed concerning the ability of antibody to limit viral excretion from throat or feces. For example, Paul, Horstmann, and Niederman state, "Our data seem to support the concept that local tissue humoral barriers may be the major deterrents to infection of the intestinal tract or the pharynx with circulating antibody playing a minor part, if any at all, at this first line of defense or the integument level" (5). Similar doubts were also expressed by Fox (6).

Following early re-infection experiments in chimpanzees, in which fecal viral excretion was clearly shown to be limited by a previous infection with homologous poliovirus (7), similar limitation of fecal and of pharyngeal virus excretion by previous homologous alimentary infection ("natural immunes") has now been repeatedly confirmed in human beings exposed to naturally occurring as well as artificially attenuated strains (6, 8, 9). Extensive field data with attenuated strains are now also available (10). Although Howe has recently shown a correlation of serum antibody

[1] Aided by a grant from the National Foundation, Inc. It is a pleasure also to acknowledge the technical assistance of William D. McClaskey, Virginia Prinn, Vincent Talbert, and Naomi Taylor.

level, induced by active immunization with formalinized vaccine, with duration and amount of pharyngeal and fecal virus excretion in virus feeding experiments in chimpanzees (11), Paul, *et al.* (5) in their studies in human beings have confirmed the inhibitory effect of pre-existing active immunity on viral excretion, but conclude that there is a greater inhibitory effect of previous homologous infection than of previous vaccination with formalinized vaccine, irrespective of antibody level. Most recent studies have emphasized the ineffectiveness of commercial poliomyelitis vaccine in preventing fecal viral excretion in vaccinated individuals, but quantitative data on level of serum neutralizing antibody has usually not been presented (12 to 16). Similarly, observations on the effects of passive immunization in human beings have been limited (17), and suggestive positive observations in confirmation of Howe's results (11) have been obtained only in relation to pharyngeal excretion (18). In our experiments with passive immunization in chimpanzees, levels of neutralizing antibody of 1:250 were shown to have little if any effect on fecal virus excretion, but the possible effect of high levels was not explored (3).

In several earlier studies we attempted to demonstrate the role of humoral antibody in inhibiting or modifying the development or movement of poliovirus in infected primate hosts. For this purpose the parenteral injection of hyperimmune serum or human gamma globulin has made possible the production of uniform serum antibody levels in the experimental hosts. Passive immunization has also led to results which could be related only to the level of specific serum antibody achieved, rather than to more complicated effects of active immunization.

The earlier reports in this series have presented evidence concerning the following effects of passive antibody:

a. The prevention of paralytic poliomyelitis after inoculation parenterally (1), or by virus feeding (2, 3).
b. The prevention of viremia after virus feeding in chimpanzees (3).
c. The modification of the active immune response after virus feeding (3), and
d. Failure at levels of 1:250 to inhibit fecal virus excretion in chimpanzees after virus feeding (3).

The present report concerns itself with experiments with virulent strains in chimpanzees, designed to extend the earlier studies of the effects of antibody on virus excretion. However, in the studies to be described the excretion of virus in throat secretions as well as in feces has been analyzed, and viral and antibody levels have been determined with tissue culture methods not available at the time of our earlier studies. The results of this study indicate that relatively low levels of passive anti-

body, as measured by serum neutralizing antibody, may have a powerful inhibitory effect on viral excretion from the throat, whereas an inhibitory effect on fecal virus excretion is only suggested at serum antibody levels as high as 1 : 500.

MATERIALS AND PROCEDURES

Viruses used. In the experiments to be reported two virulent strains were used, the Mahoney strain (Type I) and the Wallingford strain (Type II). The Mahoney strain is well known, and its invasive properties in chimpanzees have been reported previously (20). The Wallingford strain (Type II) was described in a previous report (2), and data were also presented relating to its behavior in chimpanzees (3, 20). Virus fed to chimpanzees consisted of a large pool of spinal cord suspension (K489) containing both strains which, however, were present in different concentrations. The Wallingford strain was present in high concentration, which titered $10^{-5.9}$ per ml. by intracerebral inoculation in rhesus monkeys and $10^{-6.0}$ in tissue cultures of monkey kidney cells. The Mahoney strain was present as a contaminant in the pool, at a considerably lower concentration, namely $10^{-3.0}$ TCID 50 per ml. The use of double infection made possible a study of the degree of interference in the lower alimentary tract, and of the relative susceptibility of throat and lower alimentary tract.

Inoculation of chimpanzees. All chimpanzees were selected for size and physical condition, and further conditioned for a period of several weeks to eliminate intestinal infections and to establish a satisfactory standard diet, consisting of a monkey biscuit (D&G)[2] and moderate supplements of fruit and vegetables. At the time of virus feeding, all animals were in excellent condition, and varied in weight from 14 to 28 pounds. Experimental groups were divided so that the average weight in each group was close to 20 pounds. Animals were maintained in individual cages throughout the experiment.

In the previous studies, a total of 21 immature chimpanzees were fed on one occasion with 5 ml. of a monkey spinal cord suspension containing 50,000 tissue culture doses of the Wallingford strain, by means of a blunt-tipped syringe. Three were fed with a similar dose of the Mahoney strain. In the studies to be described 17 additional chimpanzees, recently received from Africa, were infected in similar fashion, but with a mixture of both strains. The oral feeding insures contact of virus with the oral and pharyngeal mucous surfaces. Two animals died one week after virus feeding of accidental causes, namely cardiac tamponade and accidental hanging.

Passive antibody preparation. Twenty-four hours prior to virus feeding, all but four chimpanzees received an intramuscular injection of human serum, containing a high titer of Type II neutralizing antibody. Injections into muscles of calf and thigh varied from 0.04 ml. to 4 ml. per kilogram of body weight, depending on the experimental group.

The serum pool was obtained from children and adults in Middletown, N. Y. in 1952, and was used because of the high titer of the Type II component, as well as the availability of a sufficient quantity to make possible the injection of large amounts. Since the estimate of circulating serum in recipient animals was approximately 50 ml. per kilogram of body weight, the largest dose of passive serum antibody (4 ml.) was

[2] Dietrich and Gambrill, Inc., Frederick, Md. This biscuit, which contains 18 per cent protein, is prepared principally from wheat, soy bean, skim milk, alfalfa, and vitamin supplements.

TABLE I

Relation of Passive Antibody Titers in Chimpanzees to Titers of Injected Hyperimmune Human Serum

Virus Type	Neut. Ant. Titer of Serum Pool	Neut. Titer of Chimp Serum after Following Doses per Kilogram					
		Estimated			Observed		
		4 ml.	0.4 ml.	0.04 ml.	4 ml.	0.4 ml.	0.04 ml.
I	2,000	160	16	<4			<4
II	4,000	320	32	4	500	64	8
III	1,000	80	8	<4	16	4	<4

calculated to be subject to a dilution factor of 1 in 12, assuming simple dilution in blood plasma. Serum titers determined the day following injection of passive antibody were in fact somewhat lower than titers estimated on this basis (Table I), indicating distribution to other body fluid compartments. Table I also shows the specific antibody titers present in the pool, as determined by tissue culture neutralization tests.

Collection of specimens. Throat swabs around the tonsillo-pharyngeal area were taken daily for two weeks, beginning on the day after virus feeding, and subsequently on the 17th and 29th days. Swabs were immediately placed in 2 ml. of Hanks' balanced salt solution at pH 7.4, manipulated by repeated squeezing in the fluid to facilitate virus elution from the swab, and then stored at −30°C. Before assay of throat swab suspensions for virus, the suspension was thawed and clarified at 10,000 r.p.m. for 30 minutes.

Only freshly shed fecal specimens were collected from individual animals on the following days after virus feeding: 2, 3, 4, 5, 6, 12, 29. These were frozen and stored at −30°C. until ready for further treatment. Prior to virus assay, fecal specimens were diluted at 1:10 in balanced salt solution, clarified at 10,000 r.p.m. for 30 minutes, and ether-treated at 4°C. overnight.

Blood was collected for serum, from animals receiving 4 ml. and 0.4 ml. per kilogram doses of hyperimmune serum, several minutes before virus feeding, and on the 13th, 17th, 29th and 77th days. Animals receiving 0.04 ml. per kilogram, and control animals, were additionally bled daily from the 6th to the 12th days.

Viral assay. The isolation and titering of virus in throat secretions, fecal specimens, and serum of chimpanzees was carried out in both monolayer and suspended cell tissue culture systems, but in both systems primary trypsinized monkey kidney cell suspensions were grown in test tubes with a fluid medium previously described (21). The medium contains Hanks' balanced salt solution, lactalbumin hydrolysate, crystalline bovine albumin, and penicillin and streptomycin for antibacterial purposes.

Specimens to be tested were inoculated in 0.1 ml. amounts into standing 2 x 15 cm. test tubes containing monolayer cultures of 4 to 6 days growth with 3 ml. of medium in which a continuous sheet of cells of approximately 5 cm^2 was present. In the special case of fecal suspensions, the inoculum-containing medium was discarded, after 30 minutes were allowed for viral absorption, at 20°C., and replaced with fresh medium. Specimens were not considered as "negative" until at least 10 cultures, in 2 or more tissue culture "runs", had failed to yield specific cytopathogenic effects. Specimens yielding virus in the majority of pre-grown cultures were subjected to virus titration by means of a procedure similar to that described by Salk, Youngner and Ward

(22), but with endpoints read by means of cytopathogenic effects rather than by metabolic inhibition. Titrations were done in duplicate in two-fold steps, with and without type-specific serum, so that specific inhibition of viruses could be established directly upon each specimen tested, at the time of the test. Virus typing, by means of neutralization tests with all three type-specific poliomyelitis antisera, separately and in combination, was performed on all viruses isolated and not initially neutralized by Type II antiserum.

Neutralization tests. Neutralization tests were performed by means of the metabolic inhibition test (22), but with serum-free medium (21), and with microscopic confirmation of endpoints. Two-fold serial dilutions from 1:4 to 1:4000 were routinely employed. Duplicate tests at later dates were performed on all serum specimens, and in most cases agreed closely with the previous test.

RESULTS AND DISCUSSION

Only four animals experienced substantial concurrent infections of the lower alimentary tract with Type I and Type II virus, as determined by viral isolation studies. Two additional animals exhibited fecal Type I virus on one day only, whereas Type II virus was present over a longer period and in higher concentration. No Type I virus was isolated from the upper alimentary tract, possibly because of the low viral dose. Most

TABLE II

Type II Viral Excretion and Mean Neutralizing Antibody Titers in Passively Immunized Chimpanzees after Poliovirus Feeding

(High passive antibody dose, 1 day before virus feeding)

Chimpanzee		Days after Virus Feeding and Titers															Weeks		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	17	4	11	18
57-1	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Fecal Virus			2.0	2.0	2.5	3.5	3.5							2.0		3.0		
	Antibody	500													125		64	64	250
57-4	Throat Virus		0	0	0	0	0	0	0	0	0	3.5	3.5	3.8	3.5	3.5	0		
	Fecal Virus			0	0	0	0	0							3.5		2.0		
	Antibody	500													125		125	32	64
57-14	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Fecal Virus			3.5	3.5	3.5	3.5	2.0									0		
	Antibody	500													125		64	8	<4*
57-19	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Fecal Virus			2.5	2.5	2.5	0	0							3.0		2.0		
	Antibody	500													250		125	32	32

Viral titers expressed as $\log_{10}$ dilution, per gram of excretion.

0 = No virus isolated at dilution of $10^{-1.5}$ (Tables II to X).

* Partial neutralization observed at level of 1:4, when viral dose was reduced from 100 to 30 TCID 50.

TABLE IIA

Type II Viral Excretion and Mean Neutralizing Antibody Titers in a Previously Immune Chimpanzee after Poliovirus Feeding

Chimpanzee		Days after Virus Feeding and Titers														Weeks		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	17	4	11	18
58-21	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Fecal Virus		0	0	0	0	0	0						0			0		
	Antibody	4000														2000	4000		

Viral titers expressed as $\log_{10}$ dilution, per gram of excretion.

TABLE III

Type II Viral Excretion and Mean Neutralizing Antibody Titers in Passively Immunized Chimpanzees after Poliovirus Feeding

(Moderate passive antibody dose, 1 day before virus feeding)

Chimpanzee		Days after Virus Feeding and Titers															Weeks		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	17	4	11	18
57-3	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Fecal Virus			3.5	3.5	2.5	2.5	3.0						3.0			0		
	Antibody	64													32		16	125	
57-8	Throat Virus		0	0	3.5	5.0	5.0	6.2	4.4	5.0	5.0	5.0	4.4	3.2	3.8	2.6	0		
	Fecal Virus			3.5	3.5	3.5	2.5	2.0						5.0			0		
	Antibody	32													500		1000	1000	
57-10	Throat Virus		0	2.6	5.0	3.8	4.5	3.8	5.0	4.5	5.0	5.0	3.2	4.5	2.6	0	0		
	Fecal Virus			2.5	3.5	3.5	4.0	5.0						0			0		
	Antibody	32													64		2000	2000	
57-11	Throat Virus		0	0	0	0	0	0	0	*									
	Fecal Virus			0	0	0	0	0											
	Antibody	64																	

Viral titers expressed as $\log_{10}$ dilution, per gram of excretion.

* Accidental death.

of the results dealing with the effect of antibody upon viral excretion must therefore be based upon the Type II virus.

Fecal virus excretion. As was previously found (3), doses of passive antibody which produce serum neutralizing antibody levels of the order of 1:250 or lower have little if any influence upon the excretion of virus in the feces. Only 4 chimpanzees in the present study received somewhat higher levels (Tables II to V). At first glance this average level of 1:500 seems to have had as little influence upon the infection of the lower alimentary tract as did lower levels. An indication of reduction of

TABLE IV

Type II Viral Excretion and Mean Neutralizing Antibody Titers in Passively Immunized Chimpanzees after Poliovirus Feeding

(Low passive antibody dose, 1 day before virus feeding)

Chimpanzee		Days after Virus Feeding and Titers															Weeks		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	17	4	11	18
57-9	Throat Virus		0	2.1	2.8	4.5	5.0	5.0	5.0	5.0	4.5	5.0	5.0	3.8	3.8	5.0	2.6		
	Fecal Virus			3.5	3.5	4.0	4.5	3.0						3.0			4.0		
	Antibody	8													8		32	64	
57-12	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Fecal Virus			0	3.5	3.0	2.0	2.0						2.0			3.0		
	Antibody	8													4		4	64	
57-13	Throat Virus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.0		
	Fecal Virus			2.0	0	0	0	0						2.0			3.0		
	Antibody	8													4		<4	64	
57-17	Throat Virus		0	2.6	3.8	5.0	5.0	5.0	4.5	4.5	3.8	4.5	2.6	2.6	0	0	0		
	Fecal Virus			2.5	3.5	2.5	3.5	4.0						3.0			3.0		
	Antibody	8													125		1000	500	

Viral titers expressed as $\log_{10}$ dilution, per gram of excretion.

TABLE V

Type II Viral Excretion and Mean Neutralizing Antibody Titers in Control Chimpanzees after Poliovirus Feeding

(No passive antibody)

Chimpanzee		Days after Virus Feeding and Titers															Weeks		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	17	4	11	18
57-6	Throat Virus		0	0	2.6	2.6	5.0	3.8	5.0	4.5	5.0	5.0	4.5	4.5	4.5	2.6	0		
	Fecal Virus			3.5	3.5	3.5	2.0	4.0						3.0			2.0		
	Antibody	<4													4		64	64	
57-7	Throat Virus		2.6	3.2	0	4.2	3.8	4.5	4.7	4.5	4.5	5.0	3.8	3.8	3.8	2.6	0		
	Fecal Virus			3.5	3.5	3.5	4.0	4.0						3.5			2.0		
	Antibody	<4													16		32	250	
58-1	Throat Virus		3.5	4.0	3.5	5.0	3.5	2.6	3.5	*									
	Fecal Virus		4.0	3.5	4.5	4.5	4.5	3.5	4.5										
	Antibody	<4						4											
58-2	Throat Virus		0	4.0	5.0	4.5	5.0	5.5	4.5	5.0	3.0	2.6	2.6	0	2.6	0	0		
	Fecal Virus		3.5	2.5	4.5	5.5	5.5	4.5	5.5	4.5	4.5	4.5	4.5	3.5	3.5	2.5	0		
	Antibody	<4						<4	8								1000	125	

Viral titers expressed as $\log_{10}$ dilution, per gram of excretion.

* Accidental death.

total virus excretion in this group is present, however, although the number of animals is small (Table VI). Fortunately, the considerable irregularity of fecal virus excretion observed in a few previous experiments with the Wallingford virus (20) did not appear in this group of chimpanzees. Although the effect of passive antibody on fecal virus excretion is not striking, the results tend to support the correlation between serum antibody and quantitative excretion of fecal virus observed by Howe in actively immunized chimpanzees (11).

Pharyngeal virus excretion. In contrast with the small effect of passive antibody upon fecal virus excretion, a profound effect upon pharyngeal virus excretion was observed (Tables II to V). Table VII summarizes the data, which indicate that a marked inhibitory effect on total virus excretion, as well as on duration of excretion, has occurred in the group with a serum antibody level of 1:500. The single animal in this group with detectable levels of virus in throat swab extracts also showed a delay of 9 days after virus feeding before virus could be isolated from the throat.

TABLE VI

Effect of Type II Passive Antibody on Excretion of Type II Poliovirus in Chimpanzees after Single Virus Feeding

A. In Feces

Passive Serum Antibody Level at Time of Virus Feeding	500	64	8	0
No. Chimps	4	4	4	4
No. Infected	4	3*	4**	4
No. Days Tested†	27	26	28	26
No. Days Virus Isolated	19	17	23	25
No. Days Virus Titer > 1:500	9	12	16	21
% Days Virus Titer > 1:500	33	46	57	81

† Includes the following days only, after virus feeding: 2, 3, 4, 5, 6, 12 or 13, 28.

B. In Throat

Passive Serum Antibody Level at Time of Feeding	500	64	8	0
No. Chimps	4	4	4	4
No. Infected (Fecal Virus)	4	3*	4**	4
No. Days Tested†	56	49	56	49
No. Days Virus Isolated	5	24	24	43
No. Days Virus Titer > 1:500	5	21	20	34
% Days Virus Titer > 1:500	9	43	36	69

† Includes the following days, after virus feeding: 1 to 12, 13, 17.
* Uninfected animal died on 7th day by accidental hanging.
** One animal died on 8th day, following cardiac puncture.

TABLE VII

Comparison of Effect of Passive Antibody on Virus Excretion in Throat and in Feces (Data from Table 6)

Passive Antibody Level	No. Chimps	Fecal Excretion		Throat Excretion	
		No. Excreting	% Days Virus Titer >500	No. Excreting	% Days Virus Titer >500
500	4	4	33%	1	9%
64–32	4	3	46%	2	43%
8	4	4	57%	2	36%
Control	4	4	81%	4	69%
	16	15		9*	

* All animals showing throat excretion also showed fecal virus excretion.

A similar delay in the appearance of fecal virus also occurred in this animal (Table II). One additional animal (57-13, Table IV), showed a delay of 4 weeks in the appearance of throat virus, although virus had appeared sporadically in the feces beginning 2 days after virus feeding. At the time of appearance of virus in the throat the passive serum antibody had disappeared, but was later followed by an active antibody response. In all other positive animals virus was present in the throat on the 3rd or 4th days after virus feeding.

In addition to the effect of high levels of passive antibody, it appears that lower levels probably also exerted an inhibitory effect (Tables III, IV and VII). In addition to the evidence of quantitative reduction of excretion (Table VII), it can be observed that 3 of the 7 infected animals with passive serum antibody titers of 1:64 to 1:8 excreted virus in the feces, but not in pharyngeal excretions (Tables III and IV).

In a recent study, Paul, *et al.*, (5) were unable to demonstrate an inhibition of viral excretion in the throat in individuals possessing a serum antibody level of 1:64 after active immunization with formalinized vaccine, and subsequently fed attenuated poliovirus. "Natural immunes" under otherwise similar circumstances, however, failed to excrete virus. These findings are not necessarily in conflict with ours, since a much larger viral dose was fed in the human cases, and quantitative details of amount and duration of virus excretion are lacking. However, it is clear the "natural immunes" resist reinfection of the throat under conditions of antibody level and virus dosage which result in viral excretion in individuals immunized with formalinized vaccine. As indicated below, in relation to the effect of passive antibody on the active antibody response, resistance may depend on both humoral antibody as well as non-humoral factors.

Inhibition of active antibody response

In our previous study of the role of passive antibody in chimpanzees after virus feeding, it was found that passively immunized animals exhibited a reduction of the expected active antibody response following virus feeding. Moreover, 2 animals (J950, K359) receiving large doses of passive antibody failed to exhibit any active antibody response, although they excreted virus in the feces. The present study extends our earlier data on this point. In animals receiving the highest dose of passive antibody, resulting in serum antibody levels of 1:500, there was an absence of the high antibody response (1:1000 and over) which occurred in half of the animals receiving lower doses of passive antibody. In the high dose group, there was also one animal with no active antibody response, although virus was present in the stools. In this animal the decline of passive antibody was followed from a level of 1:500 to a level of <4 eighteen weeks later. The estimated half-life of passive antibody in this animal was 12 days, as previously found. The complete inhibition of active antibody response in this animal, despite alimentary infection, confirms a similar result in 2 out of 6 chimpanzees previously challenged in the same way after induction of a passive serum antibody level of 1:300 to 1:500.

Data on the complete inhibition of active antibody response by means of passive antibody, based on our entire experience with Wallingford virus, are summarized in Table VIII. It seems evident that failure of antibody response in virus re-feeding experiments, or in instances of oral exposure

TABLE VIII

Inhibition of Active Antibody Response in Chimpanzees Receiving Type II Poliovirus in the Presence of High Levels of Passive Serum Antibody

Passive Serum Antibody Level	No. Animals		8–11 Week Bleedings			Non-Responders
			Expected Passive AB Titer	Observed Titers	Geometric Mean Titer	
500	Previous* 6	10	16	10, 10, 100, 100, 200, 250	43	3
	Present 4		8	8, 32, 32, 64		
64–32	Present 3		<4	125, 1000, 2000		
8–10	Previous* 3	7	<4	150, 150, 200	200	0
	Present 4			64, 64, 64, 500		
Controls	Previous* 9	12	<4	100, 150, 250, 250		
	Present 3			300, 300, 400, 800, 5000	300	0
				64, 125, 500		

* Previously published data (3).

in immune individuals, could have as one cause the blocking action of high levels of neutralizing antibody. Low levels of circulating passive antibody (8, 32, 64) did not show any inhibiting effect in our chimpanzee experiments.

The evidence indicating the role of antibody in blocking the antibody response to alimentary infection after virus feeding is of special interest in relation to the negative results obtained in our early experiments in which parenteral exposure to virus was employed (1). The observed failure of passive antibody to affect parenteral active immunization, and the well-established ability to raise serum antibody levels to very high levels by means of successive intramuscular injections of live virus (23) or of killed virus vaccine (24), indicates at first glance that the presence of humoral antibody may inhibit the active antibody response after virus feeding but not after parenteral injection of antigen. However, Perkins, *et al.* (25) have recently reported a damping of antibody response to formalinized poliomyelitis vaccine in infants possessing passive maternal antibody.

Although their findings seem to be at variance with expectations, it is possible that the effect they obtained was dependent on quantitative relations between antibody level and antigen dose which had been lacking in previous studies. For example, the most marked inhibitory effect was found with the weakest antigens in the vaccine (Types I and III), and then only in infants with relatively high maternal antibody levels (1:64 or higher). A further indication of the importance of the quantitative relation between dose of antigen and the capacity of an immune individual to exhibit a heightened secondary response comes from the recent report of Paul, *et al.* (5). They found that reinfection of individuals with attenuated polioviruses, and the antibody response to the secondary infection, is dependent on the dose of virus fed, as shown by Sabin also (26).

In summary, the available evidence indicates that humoral antibody has the capacity to inhibit an active antibody response to poliovirus antigen, but the extent of this inhibition is dependent on quantitative relations between antigen dose and antibody level. In virus re-feeding experiments, however, the evidence clearly indicates that non-humoral factors, by affecting the degree of viral multiplication in the alimentary tract, may additionally affect the active antibody response.

Relative susceptibility of upper and lower alimentary tract

In previous virus feeding experiments with the virulent Wallingford stain, substantial titers of virus were isolated from pooled samples of feces of most animals during the first month after virus feeding (3). In a later

study, 3 chimpanzees from which samples were collected on several days, or daily in one instance, during the first 10 days after virus feeding, showed considerable fluctuation of titers of daily samples (20). In the one animal tested daily for 10 days, five samples revealed traces of virus and 5 were negative, yet a titer of 1:4000 was observed in a throat swab sample on the 10th day, when the stool sample was negative. Indeed, the contents of the ileum and ascending colon on the 10th day revealed a titer of 1:500 in this animal, so that it is obvious that the titer of virus in feces is a misleading index of the susceptibility of the lower alimentary tract. Yet, these results and those observed by Sabin (26) in chimpanzee experiments with attenuated strains, have suggested to him that the chimpanzee, unlike human beings, shows a much greater susceptibility of oropharynx to infection, as compared with the lower alimentary tract. Our present study clearly indicates that this is not the case as far as virulent strains are concerned.

In titrations of virus in 27 paired individual samples of both feces and throat swabs of 4 infected chimpanzees, between one and 10 days after virus feeding, evidence was available to make a more adequate test of the point at issue than previously was possible. These animals had received no passive antibody and were non-immune. Only samples taken prior to active antibody response are included. Table IX shows the results obtained.

It is evident that viral excretion in throat secretions and in feces during the early period after virus feeding does not differ greatly in terms of frequency of positive days or height of virus titer, in this series of animals. Indeed, the advantage, if any, is with the lower alimentary tract. The groups previously described showed greater irregularity of virus excretion in the feces under similar conditions of virus exposure. It therefore seems likely that fecal virus excretion may be influenced more by inhibitory factors in the intestinal contents than by inherently lesser susceptibility of lower

TABLE IX

Relative Susceptibility of Upper and Lower Alimentary Tract to Wallingford (Type II) Virus Feeding as Indicated by Titrations of Virus in Individual Paired Daily Samples of Feces and Throat Swab Extracts

(Non-Immune Chimpanzees, 1–10 Days after Feeding)

	No. Chimps	Samples Tested	Samples Positive	Titer of 1,000 or Higher		Titer of 10,000 or Higher	
				No.	%	No.	%
Throat Extracts	4	27	24	20	74	11	41
Feces	4	27	27	25	93	16	60

alimentary tract to infection as compared with upper alimentary tract. The results of Sabin (26, 27) and the earlier results of Koprowski (28) indicated that after feeding of attenuated viruses, susceptibility of lower alimentary tract was greater than that of oropharynx. However, these investigators incorporated the virus in milk, so that viral contact with oropharynx may have been greatly limited as compared with contact with lower alimentary tract. In our experiments with virulent strains in chimpanzees, viral suspensions were placed directly in the oral cavity and opportunity for oropharyngeal contact was excellent. Viral excretion in the throat appeared to be comparable with that observed in naturally infected human subjects.

Suppression of Type I by Type II poliovirus

Four of the chimpanzees fed Type I as well as Type II poliovirus were found to be actively infected with both types. Two had no Type I antibody at the time of onset of the experiment, but showed a Type I rise in antibody between the 13th and 22nd day after virus feeding. In these 2 animals the active antibody response to the Type II infection occurred on the 9th and 12th days, respectively. Table X shows the titers of Type I and Type II virus in throat and feces of the 4 animals exhibiting dual infections, as well as the specific serum antibody levels.

In all four animals Type I virus was not demonstrable in the throat, but only in the feces. In two instances, Type I virus was present in the feces throughout the period of 26 days during which Type II virus was present as well, and titers of the 2 virus types were similar. These 2 animals had no Type I antibody at the time of first isolation of Type I virus. The other 2 animals, which had substantial levels of Type I antibody at the beginning of the experiment, excreted Type I virus for a shorter period than Type II virus.

It is of interest that the 2 animals which excreted Type II virus in the throat for periods of 16 and 26 days, respectively, yielded no Type I virus in throat swab extracts, yet fecal specimens collected at the same time contained considerable Type I virus. These findings are of interest in comparison with Koprowski's report of apparent suppression of Type II by Type I virus in feces of human volunteers (29), and with Sabin's report of complete suppression of Type III virus in a mixture of all three types fed to chimpanzees, but only irregular suppression of Type I or Type II virus in the throat; in the lower alimentary tract he observed no such evidence of interference (30).

In unpublished experiments carried out in 1949, we observed concurrent multiplication of Type I and Type II virus in the spinal cords of rhesus

TABLE X

Concurrent Type II and Type I Infection in Chimpanzees

Chimpanzee			Days after Virus Feeding and Titers														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	28	77
57-1	Throat	I		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
(High dose, passive AB)		II		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Feces	I			0	2.0	2.5	2.5	0							0	0	
		II			2.0	2.0	2.5	3.5	3.0							2.0	3.0	
	AB	I	250													250	250	250
		II	500													125	64	64
57-9	Throat	I		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
(Low dose, passive AB)		II		0	3.3	3.8	4.5	5.0	5.0	5.0	5.0	4.5	5.0	5.0	3.8	3.8	2.5	
	Feces	I				0	3.5	3.0	4.0	4.0					3.0		2.0	
		II				3.5	3.5	4.0	5.5	3.0					3.0		4.0	
	AB	I	<4													4	8	64
		II	8						8	4	<4	<4	<4	<4	8	8	32	64
57-12	Throat	I		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
(Low dose, passive AB)		II		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Feces	I			2.5	2.5	2.0	2.5	0						2.0		0	
		II			0	3.5	3.0	2.0	2.0						2.0		3.0	
	AB	I	125													250	250	250
		II	8						4	<4	<4	<4	<4	<4	<4	4	4	32
57-7	Throat	I		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
(No passive AB)		II		2.6	3.3	0	4.3	3.8	4.5	4.7	4.5	4.5	5.0	3.8	3.8	3.8	2.5	
	Feces	I			3.5	4.0	2.5	2.5	0						4.0		2.0	
		II			3.5	3.5	3.0	4.0	4.0						3.5		2.0	
	AB	I	<4											4		4	16	250
		II	<4						<4	<4	<4	4	4	8	16	32	64	250

monkeys inoculated intracerebrally with a mixture of the 2 types. In the spinal cord, as in the lower alimentary tract, an inhibitory effect of the active antibody response on viral multiplication does not occur, whereas damping of viral excretion in the oropharynx appears to occur between the 2nd and 4th weeks after virus exposure, at the time when the active antibody response reaches levels of 1:32 or over. The fact that dual infections readily occur in spinal cord and lower alimentary tract, but not in upper alimentary tract, is correlated therefore with a marked difference of the inhibitory effect of serum antibody on viral multiplication. However, in our chimpanzees, the apparent suppression of Type I virus in oropharynx was not associated with the presence of Type I or Type II serum antibody, so that an immunological inhibitory mechanism seems incomprehensible. It is conceivable that viral multiplication in the oropharynx differs from that in the lower alimentary tract and central nervous

system in that a smaller area of susceptible tissue is involved, and is completely involved in each infected animal, so that viral interference can take place.

Relation of immune phenomena to viral multiplication and spread

The presence of humoral antibody in a host prior to virus exposure, and the development of humoral antibody during the period of active infection, have obvious importance in relation to viral development and spread. Since poliomyelitis is an infection in which high concentrations of virus exist sequentially in alimentary tract, lymphatic tissues, brown fat, and central nervous system, and in some instances persist long after the development of serum antibody, the question of the role of antibody in the damping of infection is a major problem of pathogenesis.

The denial of a role of humoral antibody in relation to immunity in poliomyelitis was almost general until it was shown conclusively that immunization with killed antigen (31, 32) and more specifically with passive humoral antibody (1, 2, 3) could prevent invasion of the central nervous system. It is well-known, however, that the humoral antibody which develops in the primary stage of infection in the alimentary tract cannot prevent paralytic poliomyelitis, since after invasion of the CNS virus can multiply and cause its damaging effects without inhibitory effect of serum antibody levels of 1:64 to 1:4000, the range usually observed after natural infections. Damping of infection in the CNS, moreover, may occur in the absence of a serum antibody response, as in rhesus monkeys inoculated intracerebrally with neurotropic strains.

Nevertheless, the balance of evidence does not permit us to discount entirely the role of humoral antibody in relation to the infectious process in the alimentary tract, as distinct from the now obvious role of serum antibody in blocking viral spread to the CNS. In a recent report, for example, Paul, *et al.*, although emphasizing the importance of virus dose in determining alimentary infection, fail to consider the possible role of *antibody level* in determining such infections (5). While the present study does not exclude the role of factors other than antibody in limiting infection of alimentary tract or CNS, it indicates that serum neutralizing antibody is capable of limiting infection of the alimentary tract, providing an adequate level is achieved. Whereas low levels of antibody may limit virus excretion from the throat, much higher levels are apparently necessary to prevent or limit infection of the lower alimentary tract or CNS when these tissues are directly exposed.

The ability of passive antibody to inhibit the antibody response to alimentary infection is also of importance in indicating that a "ceiling" level

of antibody response in individuals or populations is determined not only by resistance to reinfection but by blockage of antigen spread from sites of multiplication in the lower alimentary tract to deeper antibody forming sites. This limitation of level of antibody response after repeated virus exposure and infection of lower alimentary tract is in decided contrast to the much higher "ceiling" of antibody response to repeated parenteral immunization with live virus (23), or with formalinized vaccine (24). In view of the existence of factors which may prevent infection with live virus vaccines, such as interference between poliomyelitis virus types, or by alimentary viruses other than poliovirus (33), and of the possible failure of reinforcement of antibody levels by repeated exposure to alimentary infection, it seems possible that parenteral immunization with high potency formalinized vaccine has greater potentialities of both uniform antibody response and higher level of response with repeated injection than oral live virus immunization. With high levels of serum antibody response there should then be as durable an immune response with parenteral immunization as with oral administration of attenuated live virus vaccines. However, evidence is still inadequate to permit preference of one type of vaccine over the other on the basis of greater duration of immune response.

The ability to reinfect the alimentary tract of human beings with homologous attenuated viruses, or of chimpanzees with virulent strains, in the absence of an increase in serum antibody level, has prompted Sabin (26) to conclude that viral multiplication in the alimentary tract must take place in the surface epithelium rather than in lymphoid tissue of the mucosa, as postulated by us (20, 34). He reasons that multiplication in lymphatic tissue would inevitably lead to a detectible serum antibody response. Our experiments in chimpanzees indicate, however, that the antibody response to alimentary infection is not all-or-none, but may be graded by prior presence of humoral antibody. Thus, the failure of an increase of a pre-existing serum antibody level does not necessarily indicate the absence of an active antibody response which does not increase the total serum antibody level perceptibly. In the three chimpanzees reported in this study which showed a steady decline of passive antibody from levels of 1:500, despite alimentary infection, the occurrence of a minimal active antibody response could not be excluded.

Observations with strains of low virulence support the idea that the serum antibody response is a graded phenomenon. In the absence of viremia, the response is not as high, and minimal virus excretion is associated with minimal antibody response. In some instances viral multiplication is not followed by a detectible, neutralizing antibody response, a result which may be due to inadequate sensitivity of the test.

Our postulate regarding the site of viral multiplication in the alimentary tract was based, however, on other considerations. First, the epithelia of the two known sites, namely posterior pharynx and intestine, are different except for the intimate relation of both to lymphatic tissue. In both epithelial sites lymphocytic cells may migrate into the alimentary lumen so that lymphatic "pathways" in opposite directions exist, one being the classical drainage pathway to regional lymph nodes. Our postulated multiplication of virus in lymphatic cells thus can account for virus shedding into alimentary lumen as well as into regional lymph nodes and vascular system. The presence of high titers of virus in lymphatic stations such as Peyers' patches and deep cervical lymph nodes as early as four days after virus feeding, and the absence of virus from the remainder of the wall of the ileum at that time (20), obviously raises the possibility of multiplication of virus in lymphoid cells. This issue can only be definitely settled by methods which permit visualization of virus or viral antigen within intercellular sites of multiplication.

Of special interest in relation to the mode of spread of virus to the CNS is the fact that a low level of serum antibody is associated with the prevention of CNS infection, in the presence of active viral multiplication in the lower alimentary tract. It could be argued that a viremic route to the CNS from the throat is not necessarily supported by the protective action of low antibody levels, since such antibody levels appear to suppress viral multiplication in the oropharynx. However, in relation to infection in the lower alimentary tract, which is unsuppressed by low antibody levels, the failure of invasion of the CNS can best be explained by the ability of low levels of serum antibody to block the viremic route of viral spread. On the other hand, Sabin's alternative hypothesis (26) of nerve fiber spread to the CNS from the lower alimentary tract is difficult to reconcile with our findings in chimpanzees, in which fully active viral multiplication in the lower alimentary tract is present in animals protected from CNS invasion by low levels of passive serum antibody.

SUMMARY

1. Low levels of serum neutralizing antibody are associated with limitation of excretion of virus from the throat. Moreover, in this study serum levels of passive antibody of 1:500 appeared to inhibit such virus excretion completely in chimpanzees, although one animal began to excrete virus after the passive antibody level had fallen.

2. Low levels of serum neutralizing antibody resulting from immunization with passive antibody, fail to affect significantly the excretion of fecal virus. An inhibition of fecal virus excretion is suggested in chimpanzees

with serum antibody levels as high as 1:500. It is obvious that absolute immunity of the lower alimentary tract does not exist, as has been shown repeatedly since 1950 (7). The fact that severe reduction of fecal virus excretion, or complete inhibition, may follow a prior infection with homologous virulent or attenuated polioviruses, in the presence of low serum antibody levels, does not exclude an inhibitory role due to high antibody levels.

3. Evidence is presented which indicates that high (but not low) levels of passive serum antibody can inhibit an active antibody response in animals infected by virus feeding, and excreting fecal virus. This finding clarifies to some extent the well-established fact that prior homologous infection can prevent an antibody response to a reinfection.

4. Evidence from this study, and a previous one (20), in which virulent strains were fed to chimpanzees, does not support the claim of Sabin (26) that the chimpanzee differs from human beings in having a higher susceptibility to throat infection as compared with lower alimentary infection. The moderate doses of virus employed by us only occasionally produced limited infection of lower alimentary tract in animals with high infectivity of throat secretions.

5. It is now clear from the report of Paul, *et al.* (5), that the results of virus feeding experiments may be influenced by the dose of virus employed. As compared with recent studies in human beings, in which massive doses of attenuated strains are fed, our own studies employed relatively moderate doses of virulent strains. It is possible that the inhibitory effects of passive antibody which we were able to demonstrate would have been missed if larger doses of virus had been used.

6. The significance of these findings in further supporting the concept of invasion of the CNS by means of viral spread from the bloodstream is discussed.

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THYROID ACTIVITY DURING HYPOTHERMIA PRODUCED WITHOUT THE USE OF DRUGS[1]

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In the course of a series of experiments designed to determine the effects of various cerebral removals on the output of thyrotrophic hormone (TSH) from the pars distalis of the pituitary gland we tested the effects of changes in environmental temperature. In normal cats and in cats with extensive unilateral forebrain ablations it was found that exposure to cold (ambient temperature between 2.0° and 12.0°C.) causes in most instances an increase in the rate of thyroid secretion. This finding is in general agreement with the results obtained in rabbits by Brown-Grant, von Euler, Harris and Reichlin (1) and in rats by Brown-Grant (2) who used the method of estimating thyroid activity which we have employed.

During exposure of normal cats or of cats with extensive unilateral cerebral ablations to cold we have never observed any significant lowering of colonic temperature. But in one group of our chronic preparations exposure to very mild degrees of cold promptly produced hypothermia. With the exception of one cat, an animal with a low cervical transection of the spinal cord, this group was made up of six cats which had been decerebrated at a mesencephalic or upper pontile level. In them there remained rostral to the truncated brain stem an isolated "island" of tissue composed, in most cases, of the preoptic area, a ventral fraction of the diencephalon and the attached hypophysis (Figs. 1 and 2). These "islands" were neurally isolated (by several millimeters) from the remaining neuraxis, except for possible autonomic connections. We are unaware of any substantial evidence indicating that any autonomic innervation could exert any effect on the secretory activity of the glandular cells of the pars distalis or on neural mechanisms in the hypothalamus.

In the cat with low cervical cord transection and in all the decerebrate cats hypothermia uniformly resulted in prompt and complete inhibition of thyroid secretion as determined by the rate of disappearance of radioactivity from the neck following the administration of a tracer dose of I^{131}.

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METHODS

The animals and their care. In the experiments reported here only adult cats were used. They belonged to a colony all members of which are housed in individual cages in a room which provides a moderately well controlled environment. Air is moved through this room at a rate sufficient to change its content of air every three minutes; illumination is entirely artificial and on a 12 hour-on, 12 hour-off cycle; and the ambient temperature is maintained at 26.0° ± 0.5°C.

Much care is taken to assure the cleanliness of cages and cats. Any cat that fails, after operation, to engage in the usual feline care of its coat is brushed daily and bathed when necessary. All cages, feeding devices and water containers are cleaned daily. The animals are maintained on a modified commercial dog food ("Friskies," Carnation Co.) which the manufacturer provides salt-free. This diet is accepted by almost all normal cats and it is low enough in iodine content to permit satisfactory uptakes of I^{131} by the thyroid glands of our animals. Fresh tap water is kept at all times in the cages of cats capable of drinking. Animals unable to drink are given water by stomach tube.

Nursing care of chronically decerebrate cats. The basis of the chief difficulty encountered in maintaining a decerebrate cat or dog is the fact that these preparations are entirely poikilothermic. Although at very low and at very high core temperatures these animals do exhibit specific thermal responses, they are incapable of any effective regulation of body temperature in the face of environmental heat or cold (3). Moderately subnormal body temperatures do not appear to exert any harmful effects, but the development of hyperthermia is a great hazard particularly in animals which exhibit spontaneous motor activity. The core temperature of our decerebrate cats is maintained continuously at 38.3 ± 0.5°C. by means of an entirely automatic system which controls individual cage heaters and fans with reference to indwelling rectal thermocouples.

Feeding is begun soon after the animal emerges from the effects of the anesthetic. At that time swallowing occurs when any object is placed on the back of the tongue. In the course of a week or ten days tongue and mouth movements become well enough coordinated to assure rapid swallowing of a morsel placed near the tip of the tongue. In earlier experiments the cats were fed meat on the basis of this response and water was given daily by stomach tube. Recently we have adopted the practice of giving by stomach tube the regular diet (the commercial dog food mentioned above) homogenized with an appropriate amount of water.

Although the capacity to urinate and defecate returns shortly after decerebration, it is necessary to anticipate such evacuations in order to keep the animals clean and dry. Accordingly the bladder and rectum of each cat is routinely emptied manually at least twice a day. Occasionally constipation or diarrhea develops and it is then necessary to treat the condition immediately.

To prevent the development of hypostatic pneumonia and decubital ulcers cats which do not spontaneously right themselves (pontile preparations) are turned from one side to the other every one or two hours. Much care is exerted to detect and treat promptly any inflammation or infection of the eyes, skin and respiratory or urinary tracts. We rely heavily and with considerable success on broad-spectrum antibiotics in coping with these exigencies.

Experience in dealing with 31 chronically decerebrate preparations has demonstrated that if diligent care is given them it is possible to maintain animals with brain stem truncation at any level between upper pons and upper mesencephalon for relatively indefinite periods of time.

Operative procedures. All surgical operations are carried out under deep pentobarbital sodium anesthesia and with rigorous aseptic precautions.

Decerebration is accomplished in one stage. After making large lateral craniectomies, which leave a narrow strip of bone at the dorsal midline, and after widely opening the dura over both hemispheres, the medial surface of one hemisphere is gently retracted, the cingular gyrus sucked away to expose the corpus callosum, and the lateral ventricle opened widely. Then all neocortex and underlying white substance are removed by applying a sucker from the ventricle *outwards.* In this way much of the overlying pia containing many branches of the middle cerebral artery is left intact. Next the sucker is applied to the hippocampus. With lateral geniculate body thus exposed the optic tract serves as a guide in sucking away the amygdaloid complex, putamen and pyriform cortex. Moving rostrally, the olfactory bulb and tract, the caudate nucleus, the rest of the striatum and pallidum and the basal forebrain area back to the preoptic area are removed by careful suction. In every case the lateral and medial geniculate bodies and part or all of the thalamus proper is also removed in this manner. In the course of these procedures a noose of silk thread is slipped around the remaining pia and arterial branches and worked down to the main trunk of the middle cerebral artery and tied as close to the origin of that vessel as possible. Bleeding elsewhere is dealt with by the application of strips of "Gelfoam Sponge" (Upjohn) or cotton pledgets wet with warm saline. After completing the same ablation in the same way on the other side, the brain stem is truncated by sucking out a transverse wedge or disc of tissue at a mesencephalic level or at the level of the diencephalo-mesencephalic junction. When it is desired to have a pontile animal the entire mesencephalic tegmentum and the rostral portion of the tectum are included in the removal. In preparing a high mesencephalic cat the wedge or disc removed includes the caudal portion of the diencephalon. Care is taken to leave intact, at the base, the pia and the arterial channels. The large cranial defects are closed by drilling small holes along each side of the strip of bone left at the midline and using them to suture the medial edge (origin) of each temporal muscle to the bone. The layers of superficial muscle and fascia of the scalp are then sutured and the midline skin incision closed by a buried continuous subcutaneous suture.

The "island" of cerebral tissue (see Figs. 1 and 2) which was left neurally isolated from the truncated brain stem was composed of preoptic areas (in whole or in part), ventral diencephalon and attached hypophysis. In some instances no tissue remained dorsal to a plane passing from the anterior commissure along the ventral edge of the massa intermedia. In one animal (cat No. 100) medial portions of the hippocampus and amygdaloid complex constituted the lateral portions of the "island."

As indicated above, one cat (No. 16) used in the experiments to be reported was subjected only to transection of the spinal cord between the 8th cervical and the 1st thoracic segments. After laminectomy and transverse section of the dura (complete except for a midline ventral strip), the cord was cut with a small knife and blunt scissors. Following this the cut surfaces separate to leave a gap of two or three millimeters; there could be no doubt about the completeness of the section at the time of operation. Its level was confirmed at autopsy 191 days after transection.

Determination of thyroid activity. The techniques we employ are modifications of those described by Brown-Grant and his collaborators (1). The radioactive iodine used is obtained from E. R. Squibb and Sons (Iodotope Therapeutic Solution, Oral) as NaI^{131}. It is injected occasionally intravenously, but usually subcutaneously, in doses of from 25 to 50 μc. Measurements of neck radioactivity by means of conventional detecting (Geiger-Mueller tube) and scaling equipment are usually begun 8 to 12 hours

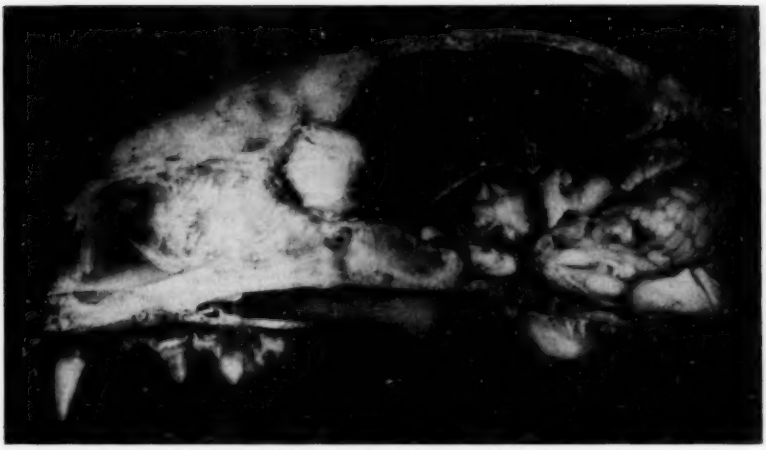

FIG. 1. View of remaining parts of the brain, *in situ*, of a decerebrate cat to illustrate method of truncation of brain stem. Note: (a) "island" consisting of preoptic area, ventral diencephalon and attached hypophysis; and (b) space left between "island" and truncated brain stem after connective tissue septum had been dissected out after fixation. At the time of operation this space was larger, for the two cut surfaces had approximated one another during the 64 days of survival.

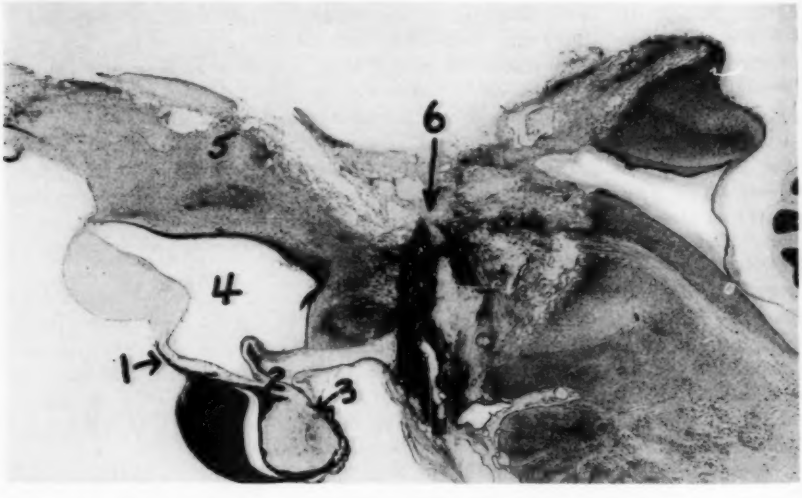

FIG. 2. A typical sagittal section from the brain of an "island" preparation. 1, intact pituitary stalk; 2, alignment of tissue along the fibers of the supraoptico-hypophysial tract in the stalk; 3, the same indicating entrance of these fibers into posterior lobe; 4, third ventricle; 5, degenerated portion of thalamus; and 6, connective tissue infiltration between "island" and truncated brain stem.

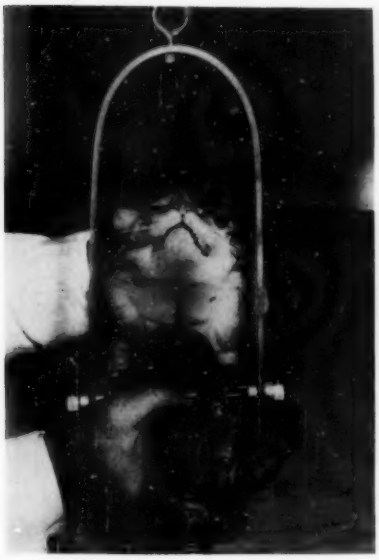

FIG. 3. Method of positioning a trained normal cat for determination of neck radioactivity.

after injection of the isotope (Fig. 3). Five to 10 one-minute counts are made every 8 to 12 hours for periods of from 10 to 20 days. After each session the one-minute counts are averaged and the figure obtained corrected for background radiation and physical decay. The results are plotted on a semi-log scale against time, zero time being the time of injection. The slope of the descending limb of the curve thus plotted describes the rate of disappearance of radioactive hormone from the thyroid gland, and is therefore a measure of the rate of secretion.

Induction of hypothermia. In our experiments hypothermia is produced in the decerebrate or spinal preparation by the simple expedient of turning off the cage heater. No premedication is necessary and none was used in any of the experiments reported here. As stated above, the animal room temperature is controlled automatically at 26.0° ± 0.5°C. When deprived of all auxillary cage heating these poikilothermic animals undergo a fall in core temperature which reaches a plateau at a level 3°–4°C. above ambient temperature. When it is desired to study the animals at intermediate degrees of hypothermia, the automatic control is set to maintain cage heating at the desired level. It may be mentioned here that one cat, No. 100, was studied before we developed automatic controls of core temperature. Consequently, at any level of environmental temperature her core temperature varied considerably (Fig. 6).

RESULTS

Survivals of the experimental animals. The low cervical spinal cat (brain intact) was maintained in excellent health up to the 191st postoperative day, when she was sacrificed. She was subjected to hypothermia on the 60th, 78th and 90th days of her survival.

The average survival of the six decerebrate cats used in this study was 138 days. Their individual survivals were 41 (No. 100), 56 (No. 114), 104 (No. 102), 140 (No. 101), 209 (No. 118) and 276 (No. 111) days. Hypothermia was induced in cat 100 on the 31st and 37th days, in cat 114 on the 51st day, in cat 102 on the 48th and 52nd days, in cat 101 on the 72nd and 79th days, in cat 118 on the 48th day, and in cat 111 on the 108th day.

Neurological status of the animals. As previously reported (3) the behavioral capacities of a decerebrate cat in the chronic state depend on the level of truncation of the brain stem. Of the six animals used in these experiments two were pontile preparations, two were low mesencephalic animals and in two the truncation was at the level of the rostral mesencephalon. Since the thyroid gland of each of these, and also the gland of the low cervical spinal cat, responded in the same way to hypothermia, the level of truncation or transection of the neuraxis is a matter of indifference as regards the problem at hand (except that in each case the central intervention had rendered the animal incapable of effective control of its body temperature).

We have obtained physiological evidence that the isolated "islands" of ventral diencephalon and pituitary gland subserve certain functions in a normal manner. As regards the hypothalamico-hypophysial control of the secretion of the antidiuretic hormone, it can be said that each of these decerebrate preparations had a normal water balance in the sense that its urinary output corresponded to the amount of water administered. In no case did any signs of diabetes insipidus develop. A normal water diuresis could be obtained in each and, with the exception of cat 100, which was not tested in this regard, the intravenous injection of a small dose of nicotine or several milliliters of a hypertonic solution of sodium chloride, caused a prompt, but temporary, antidiuretic effect. It appears, therefore, that the "osmoreceptors" of the hypothalamus, although disconnected from all extra-hypothalamic parts of the central nervous system, still function in a normal manner. Similarly, it can be stated that under basal conditions the output of thyrotrophic hormone from the pars distalis was normal, for it was found that, after the first few weeks, the amount of I^{131} accumulated by the thyroid gland and its rate of release was no different than in a group of 9 normal cats similarly studied. Finally, it is of interest to note that judged on the basis of vaginal smears these cats with "islands" produce follicle stimulating hormone after recovery from the immediate effects of the operation.

Effects of hypothermia. In each of the 8 experiments carried out on the 6 decerebrate cats reduction of core temperature (deep colonic temperature) was invariably followed by a complete inhibition of the release of

radioactive hormone from the thyroid gland. This occurred regardless of the basal rate of release, which varied from 3.5 to 19.0 per cent per day. Figure 4 illustrates the results of a typical experiment. Here there was complete inhibition not long after colonic temperature fell to 35.5°C.

That this effect is not peculiar to the decerebrate cat with "island" is indicated in Figure 5, which shows the results of an experiment on cat 16 whose brain was wholly intact, but whose spinal cord had been transected between the 8th cervical and the first thoracic segments 78 days before. The operation had rendered her almost but not quite as lacking in temperature control as had decerebration in the other 6 experimental animals. The same result was obtained on each of the 3 occasions when she was subjected to hypothermia.

The threshold for complete cessation of thyroid secretion on lowering the temperature of the poikilothermic cat has not been determined with any exactitude. On the basis of the several experiments we have done, it appears to be below 37.0°C. A core temperature of 35.0° to 36.0°C. was sufficient to inhibit the thyroid completely in 6 of our experiments and probably would have done so in the other 2 (in which the temperature was lowered somewhat farther).

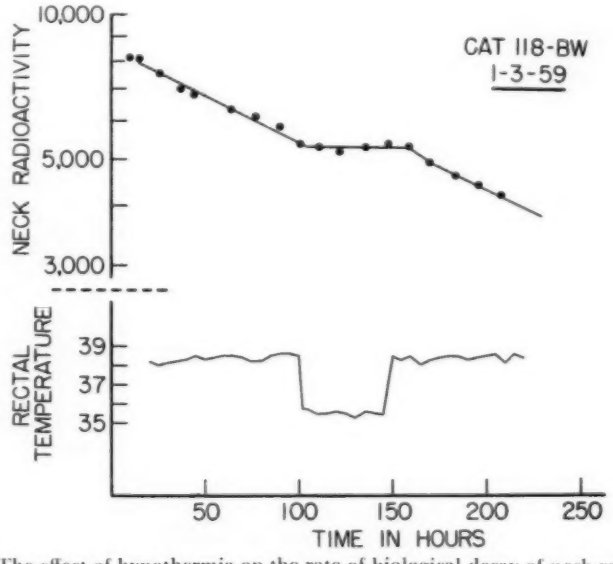

FIG. 4. The effect of hypothermia on the rate of biological decay of neck radioactivity in a decerebrate cat (No. 118) with an isolated "island" of hypothalamus and pituitary. Basal rate of decay (normal body temperature)—11 per cent per day; rate during hypothermia—zero.

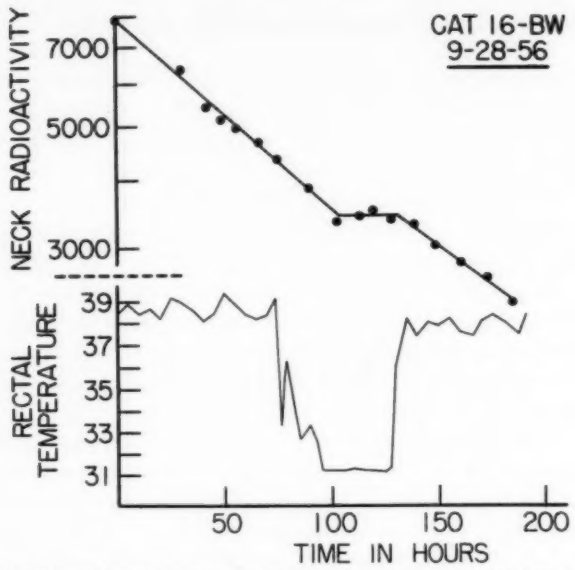

FIG. 5. The effect of hypothermia on the rate of biological decay of neck radioactivity of a cat (No. 16) with spinal cord transected between segments C8 and T1. Brain intact. Basal rate of decay—17.5 per cent per day, rate during hypothermia—zero.

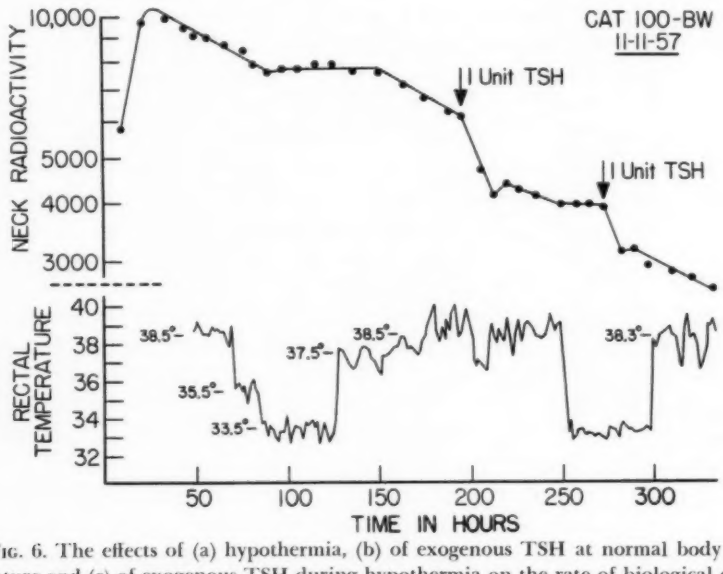

FIG. 6. The effects of (a) hypothermia, (b) of exogenous TSH at normal body temperature and (c) of exogenous TSH during hypothermia on the rate of biological decay of neck radioactivity in a decerebrate cat (No. 100) with an "island" of ventral diencephalon and hypophysis. Basal rate of decay—11.5 per cent per day; rate during hypothermia—zero; rate after giving exogenous TSH at normal body temperature—39 per cent per day; rate after same dose of TSH during hypothermia—42 per cent per day.

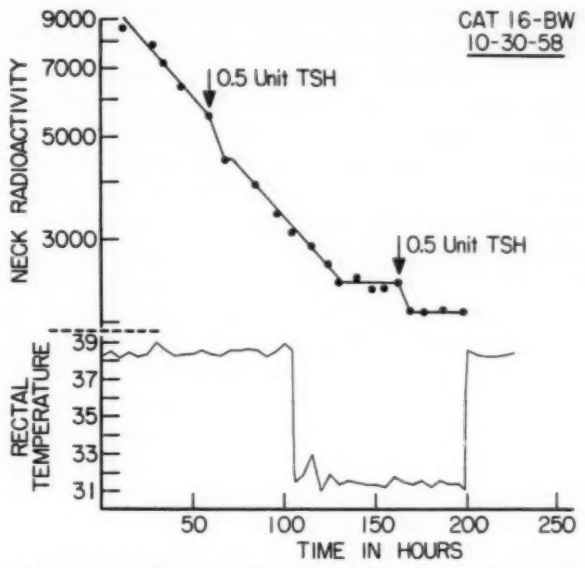

FIG. 7. The effects of the administration of the same amount of exogenous TSH on the rate of biological decay of neck radioactivity in the high spinal cat (No. 16) at normal body temperature and during hypothermia. Basal rate of decay—22 per cent per day; rate after TSH at normal body temperature—50 per cent per day; rate during hypothermia—zero; rate after TSH during hypothermia—63.5 per cent per day.

That the results depicted in Figures 4 and 5 are due to central effects, i.e., to effects on hypothalamus or pituitary or on both structures, and not to decreased sensitivity of the target organ to endogenous TSH was indicated quite clearly by the results of the experiment illustrated in Figure 6. In that experiment and in 4 other experiments on 3 other decerebrate cats, the response to a standard test injection of exogenous TSH was always greater during hypothermia than during periods when core temperature was at a normal level. In only one experiment was the reverse true and in that case the difference was insignificant. In the two experiments on the spinal cat in which the response of the thyroid gland to exogenous TSH was tested, the rate of release of radioactive hormone was also greater during hypothermia than during the control period (Fig. 7).

DISCUSSION AND SUMMARY

Although we cannot at present give any satisfactory explanation of the results obtained in these experiments, several possible mechanisms whereby hypothermia may exert its effect on the thyroid gland are worth consideration.

The first of these that came to our minds, namely, that in hypothermia the thyroid gland simply fails to respond to endogenous TSH, appears to be disposed of by the results obtained when exogenous TSH was given. The fact that in these preliminary experiments the threshold of the thyroid gland to TSH was apparently lowered by hypothermia is in itself an intriguing phenomenon which warrants further investigation.

In view of various lines of evidence that there is a hypothalamic control of the secretion of thyrotrophic hormone we next considered the possibility that hypothermia decreases hypothalamic excitability. Attempts were made to study the activity of the supraoptico-hypophysial system with the idea in mind that, if this system could be shown to react normally during hypothermia, we might provisionally assume that other hypothalamic systems are reactive under this condition. The interpretation of the results of experiments designed to test the activity of the system which regulates the output of antidiuretic hormone is complicated by the well known effects of hypothermia on renal function (4). We have not been able to produce a water diuresis in our cats during hypothermia.

There is, of course, the possibility that a low body temperature acts directly upon the pituitary gland itself. On the basis of the results of the present experiments we cannot provide any information as to whether hypothermia does have any such effect.

A possibility that demands serious consideration is that the inhibition of thyroid secretion during hypothermia is simply the result of an increased "feedback" action of thyroid hormone on the hypothalamus or the pituitary gland or on both. It is not unreasonable to suppose that in hypothermia there is a decreased utilization (and perhaps a decreased renal excretion) of thyroid hormone, since metabolic processes in general appear to be decreased. Further, what is known about the effects of hypothermia upon hepatic (5) and renal (6) functions tends to support such an hypothesis. Finally, we have some evidence (unpublished) that simple dilution of the blood during hypothermia results in at least a temporary resumption of thyroid activity.

The suppression of secretion of thyroid hormone, which occurs when body temperature as a whole is lowered, stands in sharp contrast to the fact, supported by a great mass of experimental evidence, that when the normal mammal is exposed to environmental cold one of its thermal responses is an increase in thyroid secretion. There is every reason to believe that this response is the result of an augmentation of the output of TSH brought about by an influence, neurohumoral in character, which emanates from the central temperature regulating mechanisms located in the hypothalamus. Since the classical contribution of Richet made sixty years ago,

physiologists have recognized that environmental cold may activate the central nervous mechanisms concerned with heat conservation and thermogenesis either reflexly, through stimulation of peripheral cold receptors, or centrally by cooling the arterial blood reaching the brain. It is our impression that the bulk of evidence now available suggests that the reflex component is more important than the central in determining the bodily responses which allow the homoiothermic mammal to maintain its core temperature when exposed to low environmental temperatures. In this connection it is interesting to note that with the rates of cooling used in these experiments we have never seen the slightest indication of an increase in thyroid secretion on lowering the temperature of the body as a whole (including the temperature of the blood supplying the brain) in cats made poikilothermic by decerebration or by low cervical cord transection. In the former, the hypothalamus, with its central machinery for temperature regulation and the attached pituitary gland were intact, but they were deprived of any afferent connections with peripheral cold receptors; in the latter the entire brain was intact, but the afferent input from cold receptors was confined to the parts whose innervation is provided by certain cranial nerves and the dorsal roots of the cervical segments. It is perhaps of some interest to raise the question whether the inhibitory effect of hypothermia on the secretion of TSH by the pars distalis of the pituitary glands of our cats was one that was enhanced by the massive reduction or complete exclusion of afferent impulses from peripheral cold receptors.

The experiments reported here should be regarded as entirely preliminary in nature. Certainly no definite conclusions can be drawn as regards the actual mechanism of action of hypothermia on the secretion of TSH. Our results to date, however, appear to indicate: (1) that animals rendered poikilothermic by decerebration or by low cervical transection of the spinal cord may be valuable tools in studying the effects of hypothermia without the complications which attend the use of drugs as aids in inducing a lowering of body temperature; (2) that in cats devoid of any effective temperature regulation a relatively mild hypothermia causes a cessation of thyroid secretion; and (3) that the reduction in thyroid activity is due to an effect upon an intracranial mechanism.

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HYPERGLYCERIDEMIA AND LIPEMIA RETINALIS IN HYPOPITUITARISM

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The term hyperlipemia has been variously used to describe increases in total serum lipids (1), esterified fatty acids (2), and serum triglycerides sufficient to produce lactescent serum (3). More accurate terminology has recently been suggested (4). Thus hyperglyceridemia is reserved for those states in which the serum triglyceride fraction is high relative to cholesterol level, and the serum is generally lactescent in the post-absorptive state. Hypercholesteremia denotes a rise in cholesterol relative to triglyceride, and "mixed hyperlipemia" is used when all serum lipids are elevated but are in normal proportion to each other. In the present paper, hyperglyceridemia will be used to denote lactescent serum except in those circumstances where, by common usage, the term hyperlipemia remains preferable.

Hyperglyceridemic syndromes are occasionally accompanied by lipemia retinalis in which the retinal vessels show evidence of the milky serum characteristic of these states. There is little reference in the literature to the association of hyperglyceridemia with conditions of pituitary dysfunction, and lactescent serum in hypopituitarism appears to occur very infrequently. Lipemia retinalis was first described in postpartum hypopituitarism by Leipert in 1955 (5). In this communication we report a case of hypopituitary myxedema associated with lipemia retinalis. The administration of thyrotropic hormone was followed by rapid clearing of the patient's serum with a fall in all the lipid fractions, particularly triglyceride, and disappearance of the lipemia retinalis.

CASE REPORT

A 28 year old South African Bantu male patient was admitted to the Non-European Hospital, Johannesburg, on April 29, 1957, complaining of the gradual onset of weakness, tiredness and painless swelling of the face and limbs. He had become extremely sensitive to cold during the two preceding winters and had more recently noticed progressive sexual impotence and loss of libido. He no longer required to shave, whereas prior to the onset of symptoms he was shaving every other day. There was no history of headache, vomiting or visual disturbance. Alcohol consumption had never been excessive.

Physical examination showed a pale, "puffy", well-nourished man. His height was 68 inches; weight 72 kilograms. Pulse rate was 50; blood pressure 120/90 mm. of Hg. Puffy edema of the face and limbs was noted. The skin was dry, soft and smooth. The

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heart, lungs, abdomen and central nervous system were normal. Axillary and pubic hair was scanty. The testes measured 2.5 cm. by 1.5 cm. They were distinctly smaller than normal but firm in consistency. Ophthalmologic examination showed the characteristic changes of lipemia retinalis. The retinal vessels were flat, ribbon-like and yellowish in color. Arteries and veins could only be differentiated with difficulty. The eyegrounds were yellow, superimposed on the greyish pigment of the normal Bantu fundus. The optic discs were normal and the visual fields were full.

Investigations on Admission

Radiography of the skull (Fig. 1) showed an enlarged pituitary fossa. It measured 2 cm. in its anteroposterior diameter and 0.8 cm. in depth. The anterior and posterior clinoids were well defined and there was no decalcification of the dorsum sellae. The findings were consistent with an intrasellar lesion. Radiography of the chest was normal. An electrocardiogram showed sinus bradycardia and low voltage complexes with flattened or biphasic T waves in standard leads II and III, and precordial leads V4 to V7. Basal metabolic rate was minus 27 per cent. Hemoglobin was 10.1 gm. per cent; hematocrit 30 per cent; leucocytes 5,200/cu.mm.; neutrophils 54, monocytes 4.5, lymphocytes 40.5, and eosinophils 1 per cent. Sedimentation rate (Wintrobe) was 55 mm. per hour. Serological test for syphilis was negative. Blood urea was 38 mg. per cent, serum sodium 136 mEq. per L., serum potassium 4.6 mEq. per L., serum chloride 95 mEq. per L., serum carbon

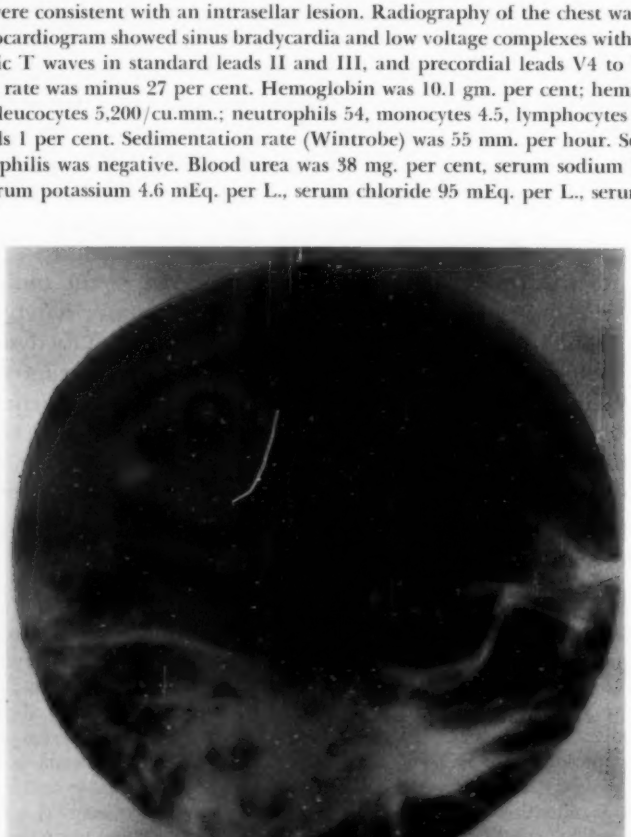

FIG. 1. Lateral radiograph of the pituitary fossa. The fossa measures 2 cm. in its antero posterior diameter and 0.8 cm. in depth.

dioxide capacity 22 mEq. per L., serum calcium 9.8 mg. per cent, plasma inorganic phosphorus 3.5 mg. per cent, fasting blood sugar 70 mg. per cent.

Liver function tests showed negative thymol flocculation and colloidal red tests, total serum bilirubin 0.8 mg. per cent, zinc sulphate turbidity 42 units, serum alkaline phosphatase 5.8 King-Armstrong units. (The thymol turbidity tests could not be done as the specimen was lactescent.) Serum albumin was 4.4 gm., and serum globulin 4.2 gm. per cent (α_1 globulin 0.3 gm., α_2 globulin 0.8 gm., β globulin 0.8 gm., and γ globulin 2.3 gm. per cent). Serum protein bound iodine was less than 1 microgram per cent, repeat estimation 1.1 microgram per cent, (normal values 4 to 8 micrograms per cent). Serum lipid studies showed total lipids 2,530 mg. per cent; total cholesterol 490 mg. per cent; phospholipids 407 mg. per cent; triglycerides 1,633 mg. per cent. The serum was markedly lactescent (Table I-A). Examination of the seminal fluid showed pus cells and epithelial cells but no spermatozoa. Urine was normal on chemical and microscopic examination. 17-Ketosteroid excretion was 2.4 mg. in a 24-hour specimen of urine. (Normal values for this laboratory are 7 to 30 mg. per 24 hours for adult males.) 17-Hydroxycorticosteroid excretion was 5.2 mg. in a 24-hour specimen of urine. (Normal values are 2.9 to 12 mg. per 24 hours for adult males.) Follicle stimulating hormone excretion was 6 to 12 mouse units in a 24-hour specimen of urine. (Normal values 6 to 48 mouse units for adult males.) Insulin sensitivity test after intravenous administration of 7 units (0.1 unit per Kg. of body weight) soluble insulin was as follows: fasting blood sugar 69 mg. per cent; ¼ hour 48 mg. per cent; ½ hour 33 mg. per cent; 1 hour 30 mg. per cent; 2 hours 44 mg. per cent.

Thyrotropin Stimulation Test (12)

(a) May 21, 1957:—Radio-active iodine (I^{131}) uptake: Neck uptake at 24 hours 7.8 per cent.

(b) May 27 through 29, 1957:—Thyroid stimulating hormone ("Thytropar", Armour) administered by intramuscular injection in a dosage of 12.5 mg. t.i.d.
May 30, 1957:—Radio-active iodine (I^{131}) uptake repeated:—Neck uptake at 24 hours 32 per cent.

Water Diuresis Test: (13)

The rate of urine flow during the second hour after ingestion of 1,440 ml. of water (20 ml. water per kilogram of body weight) was 1.0 ml. per minute. The percentage of the total water dose excreted in 3 hours was 10 per cent. After ACTH gel 80 units daily for 3 days, (June 4 through 6, 1957), the rate of urine flow during the second hour after ingestion of 1,440 ml. of water was 2.5 ml. per minute. 26 per cent of the total water dose was excreted in 3 hours. (The usual normal values for this method are a rate of urine flow exceeding 3 ml. per minute in the second hour after ingestion, and an excretion of over 40 to 50 per cent of the total water dose in 3 hours.)

Progress

Seven days after the commencement of the thyrotropin stimulation test it was noticed that the retinal abnormalities had disappeared. The patient's serum was no longer turbid. Serum lipid studies showed a marked drop in all the lipid fractions, particularly triglyceride (Table I-B).

As there was no evidence of compression of the optic nerve, nor progression of the lesion as judged by the size of the sella turcica on serial radiography, a direct therapeutic

TABLE I

	A: On Admission May 6, 1957.	B: After TSH. June 3, 1957.	C: On Replacement Therapy. June 27, 1957.	D: Normal Values for Young Urbanized Bantu (10).
Appearance	Markedly lactescent	Clear	Clear	
Total lipids: (Kunkel) (6)	2530 mg.%.	1014 mg.%.	470 mg.%.	480–770 mg.%.
Total cholesterol: (Pearson) (7)	490 mg.%.	355 mg.%.	190 mg.%.	100–250 mg.%.
Phospholipids: (King) (8)	407 mg.%.	334 mg.%.	205 mg.%.	150–250 mg.%.
Triglycerides:* (By calculation)	1633 mg.%.	325 mg.%.	75 mg.%.	0–150 mg.%.** (Peters and Man) (11)
Phospholipid/total lipid ratio.	0.16/1.0	0.33/1.0	0.44/1.0	0.31–0.41
Cholesterol/phospholipid ratio.	1.20/1.0	1.06/1.0	0.93/1.0	0.70–0.80
α-Lipoprotein (Bloomberg) (9)	3%		13%	
β-Lipoprotein (Bloomberg) (9)	97%		87%	Approximate maximum 70%
Body weight	72 Kg.	70.5 Kg.	68 Kg.	

* Triglycerides were calculated as the difference between total lipids and the sum of total cholesterol and phospholipid.

** Normal triglyceride values as stated by Peters and Man (11) are expressed as neutral fat-fatty acids.

attack on the pituitary gland was considered inadvisable. Replacement therapy was commenced with prednisone 5 mg. daily, methyl testosterone 25 mg. daily, and triiodothyronine 75 micrograms daily. Response to treatment was excellent. There was a marked increase in energy and libido. The T waves in the electrocardiogram became upright. Protein-bound iodine rose to 3.0 micrograms per cent. Failure of the protein-bound iodine to reach normal values was probably attributable to the use of l-triiodothyronine (14). Serum lipid levels reverted towards normal (Table I-C). The pituitary fossa did not alter in size, and the visual fields remained normal. The improvement in the patient has been maintained over a 2-year period of observation.

DISCUSSION

The chemical and physical properties of lactescent serum have been studied extensively. It is generally agreed that lactescence of serum is not

due to an elevation of total lipid concentration *per se*. This is well exemplified in such conditions as extrahepatic biliary obstruction and hypercholesteremic xanthomatosis (15). In these states the serum remains clear in spite of considerable elevation of the total lipid content. Since lipids remain in the serum as colloidal particles, it is the size of these particles that will determine the clarity or turbidity of serum. Particles with diameters exceeding 0.1 μ will impart a cloudy appearance to serum (16). Phospholipids, by virtue of their strong surface action, are thought to prevent coalescence of lipid fractions which would result in particles of large size. Boyd (17) and Ahrens and Kunkel (16), therefore, suggested that lactescence of serum is closely related to its phospholipid content, a low level being associated with milky serum, a high level with clear serum.

More recently, Albrink, Man and Peters (18) have stressed the importance of triglycerides, previously called neutral fat, in relation to lactescence. They stated that "an elevation of neutral fat appears to be the only obligatory change necessary for the appearance of lactescence regardless of ratios among the other lipid fractions." They considered that lactescence will invariably be present when the triglyceride content of serum rises above 20 mEq./L. (560 mg. per cent).

Lactescence of serum persisting in the post-absorptive state may occur in a variety of pathologic conditions. Since the initial description of familial idiopathic hyperlipemia by Buerger and Grutz in 1932 (19), there have been extensive studies of this disease (20 to 25). The complete syndrome of eruptive xanthomatosis, hepatosplenomegaly and abdominal colic is relatively rare. However, Wilkinson (26) believes that familial hyperlipemia represents the homozygous state for the gene, but that in the far more common heterozygous state the gene leads to high serum triglyceride levels without other manifestations of the syndrome. Havel and Gordon (27) postulate a congenital deficiency of lipoprotein lipase, the enzyme which hydrolyzes the triglyceride component of lipoproteins to fatty acids and glycerol. Other workers have reported the presence of an *in vitro* clearing factor inhibitor in the plasma of patients with familial hyperlipemia (28) and hypercholesteremia (29).

In diabetes mellitus, where a metabolic block is believed to exist both in carbohydrate utilization and in fat synthesis (30), increased mobilization of depot fat results ("transport hyperlipemia" of Thannhauser) (3). Recent work has drawn attention to the importance of the free fatty acids as a transport mechanism in diabetes (31, 32). Several workers have observed a parallelism between the severity of the diabetic state and the rise in serum lipids (2, 33, 34) although in a small group of patients hyperglyceridemia may be associated with mild diabetes (35). A rise in the serum triglyceride fraction is often the earliest manifestation of the disturbance in lipid me-

tabolism in diabetic patients with complications (36) and may precede hypercholesteremia and hyperphospholipemia.

Similarly, a "transport hyperlipemia" occurs in glycogen storage disease secondary to a defect in glycogenolysis (3). In this condition lactescent serum is associated with hypoglycemia.

Transient hyperglyceridemia may occur in acute pancreatitis and in chronic relapsing pancreatitis. The possible mechanism has been considered in detail by Albrink and Klatskin (37). The theory that there is a suppression of the pancreatic enzyme "lipocaic" (38) has not been widely accepted. Many workers believe that hyperglyceridemia results from digestion by pancreatic enzymes of adipose tissue in the omentum with liberation of fat into the blood stream (37, 39). It is not known whether pancreatitis of the chronic relapsing type follows (40) or precedes the hyperglyceridemia with which it is occasionally associated. Gross and Comfort (41) favor the view that the association is coincidental, although they consider the possibility that the same unknown metabolic or physiologic process may be responsible for the two conditions.

In lipoid nephrosis serum triglyceride and cholesterol levels are raised, often in similar proportions, and the serum is commonly lactescent. The pathogenesis of this form of hyperglyceridemia has not been established. It is probable that multiple factors are involved. Evidence has been presented to implicate increased hepatic lipogenesis (42), excessive fat mobilization (43), decreased rate of removal of lipids by the liver (44), inhibition of lipoprotein lipase activity secondary to hypoalbuminemia (45), and a combination of some of these factors (46).

The association of liver disease with hyperglyceridemia is well recognised. Considerable interest has been evoked by the studies of Zieve (47) who described a syndrome of hyperlipemia, jaundice and hemolytic anemia occurring in chronic alcoholics with fatty infiltration of the liver, usually after a heavy bout of drinking.

In pregnancy and the first few months of the puerperium significant elevation of the serum cholesterol (48), β-lipoprotein fraction (49, 50) and triglyceride fraction may occur (51), and lactescent serum has been noted in this condition since the early description by Virchow (52).

Thannhauser (3, 53) does not include either hypothyroidism or hypopituitarism among those disorders resulting in hyperglyceridemia. He considers that the few reported cases of lactescent serum and skin xanthomata in primary myxedema (54, 55, 56) are due to a chance association of myxedema and idiopathic hyperlipemia. Characteristically myxedema is associated with hypercholesteremia and hyperphospholipemia and the serum remains clear (57). Peters and Man have reported several studies on triglyceride levels in myxedema. In 1943 they stated that the concentration of

serum neutral fat was not affected by disorders of thyroid function (58). In further observations (59) they found several patients with thyroid disorders in whom neutral fat-fatty acids exceeded 6.0 mM. per L. (6.0 mEq. per L.). Most of these patients had complicating diseases such as diabetes mellitus, renal and liver disease. They concluded that increases of neutral fat rarely occur in uncomplicated disorders of the thyroid gland. However, it is interesting to note that a small group of patients had raised triglyceride levels in the absence of obvious complications, and indeed, workers from the same institution have reported a case of myxedema with lactescent serum (60). Other workers (61, 62) have found a rise in serum triglycerides in hypothyroidism, but the degree of elevation in these reports has not been impressive.

Little attention has been paid to the serum triglycerides in disorders of pituitary function. The occurrence of lactescent serum in acromegalics has been attributed to associated complications such as diabetes (63) but this question has not been resolved. Although the serum cholesterol in hypopituitarism has been widely studied (64, 65), very few observations have been reported on the response of the serum triglycerides. Peters, German and Man (66) estimated serum triglyceride levels in a series of patients with pituitary or parasellar tumors and biochemical evidence of hypothyroidism. They found that the neutral fat-fatty acid levels frequently exceeded 6.0 mM. per L. They did not comment on the appearance of the sera, but these were presumably not lactescent since the neutral fat levels were generally below the "critical level" for lactescence. They concluded that "no great significance can be attached to the irregular incidence of this disturbance (neutral fat levels above 6.0 mM. per L.) except that it does distinguish the lipid patterns of these patients in an additional respect from those of patients with primary myxedema".

Williams (67) cited a case of postpartum necrosis of the pituitary, proven at autopsy, in whom the total serum lipid concentration was 2,400 mg. per cent and serum cholesterol 262 mg. per cent. Although no further lipid studies were recorded and there is no reference to the presence or absence of lactescence, it is a reasonable inference that triglyceride levels were considerably raised.

A well-documented case of postpartum pituitary necrosis with hyperglyceridemia and xanthomatosis has been reported by Zollner (68). The serum cleared initially on a low fat diet but subsequently became lactescent again. It was then reverted to normal by the administration of thyroid hormone and has remained normal on thyroid therapy over the last 4 years (69). This patient had lipemia retinalis and is the subject of the case report by Leipert (5) to which we have already referred.

Recently Smith and Howard reported a group of patients with selective

hypopituitarism (70). Among this group was one patient with lactescent serum which was attributed to excessive mobilization of depot fat associated with severe undernutrition. Lactescence cleared during a period of gain in weight when the patient was being treated with cortisone only.

Many workers have stressed the difficulties in differentiating hypopituitary myxedema from primary myxedema (64, 65, 71) since severe myxedema *per se* can cause profound metabolic effects in all the body tissues including the pituitary and other glands; and these effects may lead to widespread endocrine failure. The patient reported above was considered to have hypopituitary myxedema on clinical and laboratory evidence. The radiological finding of an enlarged sella turcica, the measurements of which exceeded the upper limits of normal variability as judged by the criteria of Cairns and Jupe (72), was highly suggestive of primary pituitary disease, although secondary hyperplasia of the hypophysis has been reported in primary myxedema (73). The diagnosis was, we consider, established by the thyrotropin stimulation test which showed a significant increase in the 24 hour neck uptake of I^{131} (7.8 per cent to 32 per cent) following administration of thyrotropin (12, 74). The exact nature of the pituitary lesion was not established.

In view of Thannhauser's belief that the association of myxedema with lactescent serum in previously reported cases was fortuitous (3, 53), it was necessary to consider whether the present case could be an example of idiopathic hyperlipemia complicated by hypopituitarism. This was thought to be unlikely for several reasons. The dietary fat intake of the urbanized South African Bantu laborer seldom exceeds 80 gm. per day or 25 per cent of the estimated caloric intake and is largely comprised of polyunsaturated vegetable fats (75). It can reasonably be assumed that this patient developed hyperglyceridemia whilst on a relatively low fat diet and that the serum lipids returned to normal on a hospital ward diet considerably richer in animal fat content. Serum lipid studies were performed on two brothers and a sister of the patient and were normal in all respects. Furthermore, the serum lipids in patients with idiopathic hyperlipemia do not respond dramatically to hormonal therapy. Holt, Aylward and Timbres (76), using several hormones, including thyroxin, insulin, "lipocaic" and anterior pituitary extract, failed to produce any lowering of the serum triglycerides in a child with familial idiopathic hyperlipemia. Fulton (77) claimed a modest response with ACTH in a case of idiopathic hyperlipemia. Recently, estrogens have been shown to produce moderate lowering of serum triglycerides in a series of patients with idiopathic hyperlipemia, but not, however, to completely normal levels (78).

It is not possible with our present knowledge of the effects of hormones on lipid metabolism to explain adequately the mechanism of the hyperglyceridemia in this case.

Gillman and Gilbert (79, 80) have clearly indicated the complexities of the problem in their exhaustive studies on the fatty liver of endocrine origin. They were able to produce lactescent serum with intense fatty infiltration of the liver in the baboon by altering the hormonal balance between insulin, thyroxine and adrenal corticosteroids. The tendency towards hyperlipemia, for example, which follows an increase of cortisone administration will be offset by a corresponding increase in thyroxine; the effects of insulin lack may be counteracted by a depression in cortical hormone.

Several other workers have studied the influence of hormones in different experimental animals as well as in humans. These studies have been the subject of comprehensive reviews by Adlersberg (81) and Boyd and Oliver (82). The conflicting data of animal experimentation and the differences in species response have rendered it difficult to apply these results to lipid metabolism in humans. Thyroidectomy produces a striking increase in serum triglycerides in rabbits and a moderate increase in dogs (81). In man thyroid hormone exerts its influence predominantly on the serum cholesterol with minimal effects on triglyceride concentrations (3, 57 to 59). Desiccated thyroid and thyroxine have been shown to reduce serum cholesterol and β-lipoprotein levels of euthyroid as well as hypothyroid subjects (83, 84). Similar changes have been observed following the administration of a wide variety of thyroxine analogues (85 to 88).

Although our knowledge of the mode of action of thyroid hormones on the body lipids remains incomplete, considerable information has accumulated in recent years. Several workers have shown that thyroid hormone increases, and hypothyroidism diminishes, the rate of cholesterol biosynthesis in the liver in experimental animals (89, 90), and in man (60, 91). Nevertheless, the serum cholesterol level is generally depressed in hyperthyroidism and elevated in myxedema. The available evidence points to the fact that catabolic and excretory processes are slowed down in hypothyroidism as much, if not more, than biosynthetic ones (92). Thus Rosenman, Friedman and Byers (93) have shown that in rats given injections of hypercholesteremic serum, the excess of cholesterol is removed from the serum most rapidly in hyperthyroid, and least rapidly in hypothyroid animals.

Reports differ on the response of serum lipids to corticotropin and adrenal corticosteroids. Many workers have induced marked hypercholesteremia (96) and hyperglyceridemia (97) in different experimental animals with these agents. Others (98) have shown that although cortisone increases the serum triglycerides and cholesterol in normal rabbits, it will appreciably depress the hypercholesteremia resulting from the feeding of cholesterol to these animals. In man the response is more uncertain. Adlersberg, Schaefer and Drachman (99) observed a rise in serum cholesterol and phospholipids and a fall in triglycerides in a group of severely ill patients with 'collagen-

vascular' diseases. Oliver and Boyd (100) found a reduction in serum cholesterol and β-lipoproteins in hypercholesteremic patients with coronary atherosclerosis. Barr (101) produced no noticeable effect with cortisone in a group of normal subjects. Skanse, von Studnitz and Skoog (102) suggested that the discrepancy between the results of Adlersberg and associates (99) and Oliver and Boyd (100) was probably due to differences in the length of treatment in the two studies. However, it is possible that the difference in response is associated with the type of subject studied and that the "permissive" action of cortisone can result in either elevation or depression of serum lipid levels under various circumstances.

There is general agreement regarding the reduction in serum cholesterol which follows the administration of estrogens and their analogues in man (103, 104) although this hormone appears to increase serum cholesterol in rats (105) and birds (106). Androgens, on the other hand, are inconsistent in their effect, but tend to reduce α-lipoproteins and raise β-lipoprotein and cholesterol levels (107). Although insulin exerts a profound effect on all serum lipid fractions in diabetes (108), there is no definite evidence that it has any significant effect on the blood lipids of metabolically normal men (82).

Considerable interest is currently being focussed on the role of the pituitary gland in the regulation of tissue and serum lipids. The fat-mobilizing property of anterior pituitary extracts has been known for many years (109). This property has at different times been attributed to thyrotropic hormone (110, 111), adrenocorticotropic hormone (112) and growth hormone (113). Astwood (114) suggested that the pituitary may secrete a specific substance which favors utilization of depot fat. This view is supported by Rudman, Seidman and Reid (115) who were able to separate the major portion of the lipemia producing activity from ACTH and growth hormone. They did feel, however, that ACTH had a potentiating effect upon the production of lipemia. Seifter and his associates (116a) have found a lipid mobilizing substance in the plasma of several animal species, including man (116b). They have crystallized this substance, the primary function of which is to mobilize triglyceride from omental and mesenteric depots, from the plasma of cortisone treated horses (116c). Although the anterior pituitary hormone is essential for the release of this hormone (116d) there is evidence that it is stored in the posterior pituitary gland (116e). These workers consider that lipid mobilizing hormone plays a vital role in the lipemia of familial hyperlipemic and hypercholesteremic states (29), pregnancy (116f), nephrosis, and several other disorders (116g).

The dissociation of tropic functions in many patients with hypopituitarism is frequent and may result in varied and sometimes bewildering patterns (70, 117). It is therefore theoretically possible that our patient was

suffering from selective hypopituitarism and that, whereas he was grossly deficient in thyrotropic hormone, his production of lipid-mobilizing factor remained adequate or even excessive. The liberation of thyroid hormone from the dormant but potentially active thyroid gland following administration of thyrotropic hormone may have produced a complex alteration in the hormonal balance between lipid-mobilizing hormone, cortisone and thyroxine, which in some way resulted in clearing of his serum. This response to thyrotropic hormone is remarkable in view of previous studies wherein the administration of thyrotropin in guinea pigs resulted in the production of lipemia (110).

The extent of participation of the adrenal cortex in the hyperglyceridemia of our patient requires consideration since it is well established that in the experimental animal cortisone is essential for mobilization of fat (118). It is possible that the disturbance in thyroid function in our patient was considerably greater than that in adrenal cortical function, and that cortisone production was in relative excess to production of thyroxine. The resulting endocrine environment could then be compared with a particular situation created by van Zyl (119). This worker showed that the administration of cortisone to pituitary stalk-sectioned baboons resulted in a moderately intense lipemia which could be reduced by the administration of thyroxine even though cortisone therapy was continued. Our patient did not show the marked fluctuations in weight which characterized the case described by Smith and Howard (70). Clearing of the serum in their case following cortisone therapy may have been a reflection of the permissive action of cortisone.

There has been a tremendous renewal of interest in the serum triglycerides since Albrink and Man presented statistical evidence to suggest that an error in triglyceride metabolism is the main lipid abnormality operative in patients with coronary atherosclerosis (120). In consideration of the above and similar cases, the current viewpoint that thyroid hormones exert little or no effect on triglyceride metabolism in man (57) requires re-evaluation.

Lipemia Retinalis

The striking ophthalmoscopic appearance of lipemia retinalis has been fully described (121). The retinal changes are always associated with hyperglyceridemia; however, the serum may be grossly lactescent without producing manifest changes in the retinal vessels. Thus Jaffe and Schonfield (122) reported a case in which lipemia retinalis disappeared when the total serum lipid level was greater than 16 gm. per cent; McCann (123) noted clearing of the retina at a time when the total serum lipid level was rising; and Manning, White and Carne (124) observed improvement while the

TABLE II

Non-Diabetic Lipemia Retinalis

Author	Case Description	Lipid Studies	Etiology	Comment
Wagener. (127)	Boy 9 yrs. Lymphatic leukemia. Hyperlipemia after radium therapy.	T.L. 5 gm.%.	Result of radium therapy.	Normal fundus following blood transfusion.
Holt, Aylward, & Timbres. (76)	Girl 11 yrs. Hepatosplenomegaly, abdominal colic, psoriasis, skin ulceration, undernutrition.	T.L. 7–8 gm.%. Marked rise in triglycerides.	Juvenile familial idiopathic hyperlipemia.	Response to low fat diet and blood transfusion. No response to thyroxine, insulin, ant. pituitary extracts, choline, lecithin or "lipocaic".
Holt, Aylward, & Timbres. (76)	Boy 2½ yrs. Hepatosplenomegaly. Brother of Case 2.	T.L. 3 gm.%. Predominantly triglycerides. T.C. 209 mg.%. P. 305 mg.%.	Juvenile familial idiopathic hyperlipemia.	Response to low fat diet.
Bernstein, Williams, Hummel, Shepherd, & Erickson. (128)	Boy 7 yrs. Hepatosplenomegaly, xanthomatosis.	T.L. 7430 mg.%. T.G. 5430 mg.%. P. 941 mg.%. T.C. 1059 mg.%.	Juvenile idiopathic hyperlipemia.	Response to low fat diet.
Goodman, Shuman, & Goodman. (129)	Boy 1 yr. Hepatosplenomegaly, xanthomatosis, malnutrition.	T.F.A. 3115 mg.%. P. 465 mg.%. T.C. 379 mg.%.	Juvenile idiopathic hyperlipemia.	Response to low fat diet.
Kauffman. (130)	Negro male, 25 yrs. Chronic alcoholic.	T.C. 980 mg.%.	Chronic alcoholism.	Inadequate clinical and laboratory studies.
Lepard. (131)	Boy 6 yrs. Hepatosplenomegaly.	T.L. 9370 mg.%. T.G. 7636 mg.%. P. 833 mg.%. T.C. 901 mg.%.	Juvenile idiopathic hyperlipemia.	Response to low fat diet.
Grossman & Hitz. (132)	Male 55 yrs. Hepatomegaly, abdominal colic. "Fatty liver" on biopsy. Reported twice (133).	T.L. 13.13 gm.%. T.C. 1128 mg.%.	Adult idiopathic hyperlipemia.	Response to low fat diet.

Groom & Rucker. (134)	Male 59 yrs. Hyperlipemia found on routine examination. No signs.	T.L. 3584 mg.%. T.F.A. 2944 mg.%. T.C. 640 mg.%.	Idiopathic hyperlipmia.	Probably familial form. Son also had hyperlipemia.
Groom & Rucker. (134)	Male 33 yrs. Chronic alcoholic. Thyroidectomy 1 year previously.	T.L. 4740 mg.%. T.F.A. 3528 mg.%. T.C. 1212 mg.%. P. 1136 mg.%.	Chronic alcoholism.	Response to high carbohydrate diet.
Groom & Rucker. (134)	Boy 7½ months. Hepatosplenomegaly, xanthomatosis. Fat-laden cells on bone marrow.	T.L. 5242 mg.%. T.C. 448 mg.%.	Niemann-Pick disease.	Inadequate criteria for diagnosis of Niemann-Pick disease.
Dunphy. (135)	Male 47 yrs. Xanthomatosis. Fasting blood sugar 206 mg.%. No hepatosplenomegaly.	T.F.A. 5196 mg.%. T.G. 4477 mg.%. P. 810 mg.%. T.C. 693 mg.%.	Idiopathic hyperlipemia.	Response to low fat diet. Associated hyperglycemia.
Everett. (136)	Boy 3½ yrs. Epileptic. On high fat diet. No hepatosplenomegaly.	T.C. 1280 mg.%.	? diet induced hyperlipemia.	Response to low fat diet. Probably result of ketogenic diet.
Davies. (137)	Male 30 yrs. Xanthomatosis. Spleen palpable.	T.L. 5600 mg.%. T.C. 641 mg.%.	Idiopathic hyperlipemia.	Response to low fat diet.
Leipert. (5)	Female 34 yrs. Hypopituitarism. (Sheehan's syndrome). Reported twice (68).	T.L. 2056 mg.%. T.G. 1582 mg.%. P. 422 mg. %. T.C. 380 mg.%.	Postpartum hypopituitarism.	Response to low fat diet. Hyperlipemia on normal diet. Response to thyroid hormone.
Payne & Waldron. (138)	Male 36 yrs. Abdominal colic and diarrhoea. Xanthomatosis.	T.F.A. 6280 mg.%. T.G. 5786 mg.%. T.C. 643 mg.%. P. 850 mg.%.	Essential hyperlipemia.	Response to low fat diet.

T.L. = Total lipids; T.F.A. = Total fatty acids; T.C. = Total cholesterol; P. = Phospholipids; T. G. = Triglycerides.

serum triglyceride level remained unaltered. A likely explanation for this occasional lack of correlation between serum triglyceride levels and the appearance and resolution of lipemia retinalis has been offered by Laws and Harpur (125). They suggested that in lipemia retinalis the chylomicrons, which are larger and less dense than erythrocytes, move slowly and tend to be displaced towards the periphery of the vessel lumen. The vessel thus contains a fast-moving central core of red cells surrounded by a collar of slower-moving chylomicrons, and appears whiter than normal when viewed from the side. This appearance is accentuated in the presence of anemia. Therefore both a rising red cell count and a decreasing lipemia could account for clearing of lipemia retinalis. The level of triglyceride at which lipemia retinalis generally becomes apparent is not critical but varies between 1.5 gm. per cent and 5 gm. per cent. The presence of anemia in our patient probably contributed to the rather marked retinal changes which were present at a serum triglyceride level of 1.6 gm. per cent.

In 1930 Parker and Culler wrote that "no disease other than diabetes uncomplicated by treatment produces lipemia of sufficient degree to be recognized ophthalmoscopically" (126). The only exception at the time was the case reported by Wagener (127) of a 9 year old boy with lymphatic leukemia who developed lipemia retinalis following radium therapy. It was suggested that extensive destruction of reticuloendothelial tissues by radium had resulted in the liberation of large amounts of lipid into the blood stream.

A total of 16 cases of non-diabetic lipemia retinalis have now been reported (127 to 138). These are tabulated in Table II. The paucity of these reports in no way reflects the prevalence of the condition since the retinal changes may be transient and could easily be overlooked. It is likely that most cases of idiopathic hyperlipemia develop lipemia retinalis at some stage in the course of the disease.

Ten of the reported cases of non-diabetic lipemia retinalis have been in patients with idiopathic hyperlipemia. A familial history of hyperlipemia was present in the brother and sister reported by Holt, Aylward and Timbres (76) and in one of Groom and Rucker's adult cases (134). Dunphy's case (135) is of considerable interest as the patient showed clear evidence of diabetes mellitus. Lepard's case (131) also had an abnormal glucose tolerance curve. The association of mild diabetes with idiopathic hyperlipemia has been observed by several workers (3, 35). Smith (139) found 13 patients with glycosuria in a review of 95 cases of essential hyperlipemia. Vascular complications are frequent in these patients, and it is of some importance to differentiate this condition from hyperglyceridemia secondary to severe diabetes since the abnormal lipid patterns respond more readily to a low fat diet than to insulin.

The epileptic child reported by Everett (136) developed hyperglyceridemia and hypercholesteremia after the ingestion of a high fat diet over a period of several months. Lactescence and lipemia retinalis cleared after a 24 hour period of starvation. Walsh (140) cited a similar example of an epileptic child in whom lipemia retinalis was induced by a ketogenic diet.

The two alcoholic patients with lipemia retinalis (130, 134) are not well documented, and it is not possible to determine whether the hyperglyceridemia was associated with acute pancreatitis (37) or with fatty infiltration of the liver.

The diagnosis of Niemann-Pick disease in the report by Groom and Rucker (134) is open to question as clear diagnostic criteria were not presented. Although occasional examples of a mild hyperglyceridemia have been noted in patients who show marked increases of lipid in the viscera (141), there is no consistent abnormality of the serum lipid patterns in this condition.

Lipemia retinalis in primary pituitary disorders has been observed in acromegalics by Igersheimer (142) and by Walsh (140). These patients had associated diabetes mellitus which may have been responsible for the serum lipid abnormalities. Our case and the case reported by Leipert (5) are the only examples on record of lipemia retinalis occurring in hypopituitarism.

SUMMARY

1. The occurrence of hyperglyceridemia with lipemia retinalis is described in a case of hypopituitary myxedema.

2. The nature of lactescent serum is discussed and the causes of hyperglyceridemia are outlined.

3. Mention is made of the paucity of reports of hyperglyceridemia in association with hypothyroidism and hypopituitarism.

4. Relevant experimental and clinical data on the hormonal influences on serum lipids is reviewed, and its possible application to our case is considered.

5. The condition of non-diabetic lipemia retinalis is critically reviewed.

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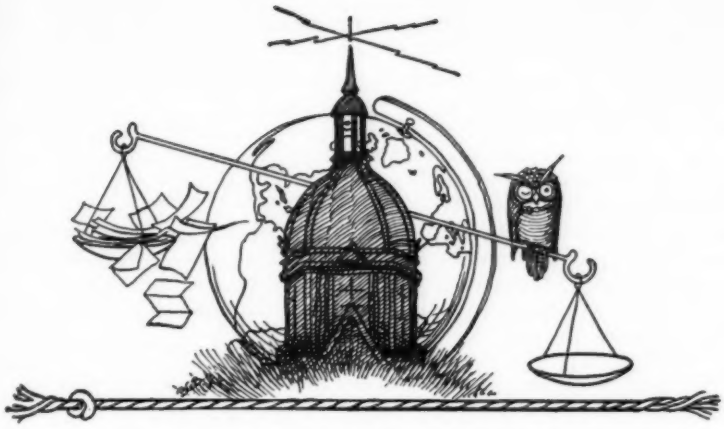

BOOK REVIEWS

Diseases of the Nose, Throat and Ear. A Handbook for Students and Practitioners. By I. Simson Hall. 467 pp., $5.00. E. & S. Livingstone Ltd., Edinburgh and London. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

This 450 page handbook is designed for the medical student and practitioner and is in its seventh edition since 1937. The text material is quite good for the group of readers for which it is intended. Several subjects are rather briefly discussed, namely: electric nasopharyngoscopy, the treatment and diagnosis of nasal and facial fractures, severe oesophageal burns and congenital anomalies of the ear. However, this book was not designed to cover all of the general field and in its present form would be satisfactory for the use of medical students and nurses.

F. I. Catlin

Current Virus Research. British Medical Bulletin, Vol. 15, No. 3, Sept. 1959. 250 pp., $3.25. Published by the Medical Department, The British Council, London, England.

The reviews published in the British Medical Bulletin during the past few years have added significantly to the availability of definitive information on a variety of subjects of medical interest, and this volume is no exception. In reviewing the many phases of research on viruses authors with international reputations have surveyed knowledge of basic virology, diagnostic techniques, and contributions to our understanding of several of the important virus diseases. Anyone interested in research or clinical problems on virus infection should read this issue.

Leighton E. Cluff

A Textbook of Medicine, Vols. I and II, 10th edition. Edited by Russell L. Cecil and Robert F. Loeb. 1754 pp., $20.50. W. B. Saunders Co., Philadelphia, Pa., 1959.

The many strides which have been made in medicine in the four years since the ninth edition have necessitated this most recent edition. As has been the custom in the past, the editors have obtained the services of the leading men in American medicine to write the sections covering subjects not previously presented. Cytomegalic inclusion disease,

aminoacidurias, agammaglobulinemia, carcinoidosis, macroglobulinemias, and hyperaldosteronism are among the newly discussed subjects.

As in the past, this textbook will provide the medical student (and the internist reviewing for his board examinations) with an encyclopedic approach to internal medicine. It will not usually provide answers to questions that continually arise in the day to day confrontation of human illness.

But, as Beecher has commented, "lacking as we do perfect memories, the fruits of the great sources of knowledge quickly spoil unless they are preserved." Therefore we must have textbooks of medicine. To be sufficiently comprehensive and authoritative, they must have multiple authorships. Primarily because of the high caliber of its authors, this textbook is better than most, and as good as any.

HENRY N. WAGNER

BOOKS RECEIVED FOR REVIEW

Attenuated Infection. The Germ Theory in Contemporary Perspective. By HAROLD J. SIMON. Forewords by RENE J. DUBOS AND WALSH MCDERMOTT. 349 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

The List Method of Psychotherapy. By ELIZABETH SHER, ELEANOR MESSING, THEODORA HIRSCHHORN, ENIS POST, ANNETTE DAVIS AND ARTHUR MESSING. With an Introduction by JACOB S. LIST, 259 pp., $7.50. Philosophical Library, New York, N. Y., 1960.

The Office Assistant in Medical Practice, 2nd edition. By PORTIA M. FREDERICK AND CAROL TOWNER. 407 pp., $5.25. W. B. Saunders Co., Philadelphia, Pa., 1960.

Steppingstones to Professional Nursing, 3rd edition. By Luella J. Morison. 389 pp., $4.75. The C. V. Mosby Co., St. Louis, Mo., 1960.

The Thyroid Gland. British Medical Bulletin, Vol. 16, No. 2, May, 1960. 174 pp., $3.25. Published by the Medical Department. The British Council, London, England.

Viral Infections of Infancy and Childhood. Symposium No. 10, Section on Microbiology, The New York Academy of Medicine. Edited by Harry M. Rose. 244 pp., $8.00. Paul B. Hoeber, Medical Division of Harper & Bros., New York, N. Y., 1960.

THE PHYSICIAN AS A WORLD CITIZEN[1]

H. VAN ZILE HYDE[2]

Thucydides, in one of those inimitable speeches he put into the mouths of others, quotes the Corinthian spokesman at the Congress of the Peloponnesian Confederacy which was held in 432 B.C. as saying, "the true author of the subjugation of a people is not so much the immediate agent, as the power which permits it, having the means to prevent it."

This poses the question: do we as physicians have power and are we using it responsibly in preventing subjugation by disease and by tyranny? Perhaps we cannot answer this question but we can examine some of the factors that bear on it.

In 1901, Dr. William Osler, in reviewing the accomplishments of medicine in the Nineteenth Century, cited as diseases that "have been remarkably controlled": smallpox, typhus, typhoid, cholera, yellow fever, plague, tuberculosis, diphtheria, pneumonia, hydrophobia, malaria, venereal diseases, leprosy and puerperal fever.[3]

A mere listing of these diseases reminds us that the control anticipated by Osler 58 years ago has not been accomplished, despite great technical achievements that he in all his optimism could not foresee.

In 1958, there were 250,000 cases of smallpox in the world. Millions die each year of the enteric diseases, the chief cause of death in the world today. Cholera kills 100,000 persons and tuberculosis several million each year. A recent report of the World Health Organization stated that "a fair estimate of the average prevalence would be that one-half to 1 per cent of the adult population in the world suffers from infectious tuberculosis."[4] Over 150,000,000 cases of malaria can be expected throughout the world this year, and it is estimated that 25,000 new cases of syphilis will be diagnosed in the United States. Fifty million persons in Africa and Asia suffer from yaws. Ten million to 15 million cases of leprosy persist.

Taking note of some other preventable diseases not on Osler's list, there are estimated to be 400,000,000 cases of trachoma and related eye diseases; 40,000,000 cases of schistosomiasis; up to two billion worm infestations and over a billion persons undernourished.

[1] Alpha Omega Alpha Lecture, Johns Hopkins University, December 17, 1959.

[2] Dr. Hyde is Assistant to the Surgeon General For International Health, U. S. Public Health Service, Washington 25, D. C.

[3] *Aequanimitas, With other Addresses to Medical Students, Nurses and Practitioners of Medicine*, "Medicine in the Nineteenth Century," Sir William Osler, P. Blakiston's Son & Co., Inc., Philadelphia, 1932, p. 239.

[4] *Tuberculosis Programme of WHO*, (mimeographed) document number WHO/TBC/Int./44, August 5, 1959, p. 5.

Whereas gross mortality rates have been reduced below 9 per 1,000 persons and infant mortality rates below 20 per 1,000 live births in some countries, in others these rates still range up to 50 and 250, respectively.

Has our stewardship been good during the past 58 years? Have we had the power to prevent subjugation by diseases and failed to use it?

Against this background and with the question of our role in mind, let us direct our attention for a few moments to the general world situation.

Two of the most significant facts of our day are: first, that two ideologies —tyranny and freedom—are locked in a struggle that can lead to the destruction of civilization or of man himself; and, secondly, that underprivileged people of the world are on the march to gain for themselves those privileges that you and I take for granted. It is, indeed, the rising of a revolution against distress and degradation.

We are all acutely aware of the ideological struggle and the force behind it. The aggressive nature of the tyranny involved is clear. The goals of communism set out by Lenin, Stalin and Khrushchev are much clearer than those of the muddled *Mein Kampf*, which so few of us read but which was followed step by step on an orderly, announced path toward the near destruction of everything in which we believed. Khrushchev confirmed his dedication to the objectives of world communism during his recent visit. No fundamental change can be recognized.

In regard to the second point, Paul Hoffman, the Managing Director of the U. N. Special Fund, and former administrator of the Marshall Plan and President of the Ford Foundation, recently summed up the situation as follows:

"One epochal event after another has crowded in on us in this twentieth century. Yet I am certain that if a historian writing, say, in the year 3,000 were asked to select the most significant event, he would have no difficulty in doing so. It would not be World War I or World War II, or the discovery of atomic energy, or even the conquest of outer space. No, I think he would point to a revolt—not a political revolt against foreign domination but a revolt of hundreds of millions of people against the continued acceptance of poverty, sickness, and illiteracy as their normal life—something to be borne patiently because no other life could be imagined. The awakening of these underprivileged people, whose forebears had slumbered peacefully through the centuries, is the truly significant and pervasive fact of our times."[5]

To be more precise, the per capita income in the United States is over $2,100 per year; that of 1,250,000,000 people averages $100 per year, and

[5] From an address before the First Conference of the Society for International Development, Washington, D. C., February 1959.

in many areas it is as low as $50. This is a crude measure of the degradation against which the mass of mankind is fighting.

For us, as physicians, it is not difficult to relate the burden of preventable disease to the social problem confronting the underdeveloped areas. It has two facets: health is an end goal in itself; it is, as well, a necessary component of a strong economy. I need not belabor these points.

How does this relate to the ideological struggle? Perhaps, it does so through the *impatience* that characterizes the revolution of the underprivileged.

Many from all walks of life and all lands have now seen the evidences of progress—in material terms—that has been accomplished under the tyranny of the Russian communist regime. This is progress under tyranny but the tyranny does not show as clearly as the progress, since as Mrs. Roosevelt has so aptly said, "freedom is not something you can describe, it must be felt." The tyranny only becomes obvious when it overflows into Korea, Indochina, Hungary, Tibet or India. The tinsel of progress glitters throughout the year.

The progress under tyranny in the Soviet Union can be appreciated by contrasting the Soviet Republics of Tadzhikistan or Uzbekistan with contiguous Afghanistan. The populations of Tadzhiks, Uzbeks and related ethnic groups are basically the same; the dry hills and mountains are the same. The border between them is but a political line, not a cultural or physical divide. To the north, the Soviet side, where on September 1, 1957, I saw every child in town at the Park of Rest and Culture celebrating Children's Day, the children are well nourished, free of disease, neatly dressed, energetic and at school. To the south, in Afghanistan and down through India and Pakistan, millions of children are ragged, semi-starved, worm-ridden and listless, with no school available. Medically, Afghanistan with a population of some 12,000,000 persons has 224 physicians, or approximately one per 52,000 persons and 1,642 hospital beds. Tadzhikistan with a population of 1,800,000 has 1,700 physicians, or approximately 1 per 1,000, and 9,200 hospital beds. Its medical school is training 300 students per class, 50 per cent of whom are derived from the indigenous population of Tadzhiks and Uzbeks.

The question at issue is—will the impatient, underprivileged of the world be able to attain the better life they seek under a free system or will they, in their impatience, fall into the trap set for them? Have we the power to effect this?

This leads us squarely to ourselves. What power do we, as physicians, have?

We have indeed very unusual power. This is the main lesson I have

learned in my years in international work. It derives from characteristics of our profession: our ideals; our technology; our constituency; and our solidarity.

Ideals have great force on the minds and in guiding the actions of men. As stated by Osler: "... By the ordinary mortal, idealists are regarded as vague dreamers, striving after the impossible; but in the history of the world how often have they gradually moulded to their will conditions the most adverse and hopeless!"[6] No other group has such an ideal for self and others, as that set for us centuries ago by Hippocrates.

I need not dilate on our *technology*. Many of us here have been eyewitnesses and participants in the drama which has included in its cast the sulfonamides, antibiotics, tranquilizers, vitamins, insecticides. We know that through our technology we have more power to influence the daily life of men, women and children than does any other group of men. Malaria, which has struck nearly 100,000,000 persons each year in India, is being eradicated there. What more profound and personal change could be brought about within a population?

Our *constituency* is vast. Our forebears have won for us the faith of mankind. The magic front has given way before science, which is now so completely enthroned in the minds of man. We have, indeed, gained a worldwide following.

I have taken the word "*solidarity*" from Dr. Osler to express the fourth source of our power. "The profession," he said, "in truth is a sort of guild or brotherhood, any member of which can take up his calling in any part of the world and find brethern whose language and methods and whose aims and ways are identical with his own."[7]

One of the most impressive phenomena on the world political scene today is the non-political relationship among the participants in the annual Assembly of the World Health Organization. Here the flow of ideas, information, intentions and desires are characterized by the friendship and rapport derived from common understanding and common motivation among physicians.

Here again is power—power to bridge conflict, to lead through the maze of contending forces to the higher goals that man is seeking.

Thus, as a citizen of the world, the physician has power—through his ideals, technology, constituency and solidarity—that he can apply to the subjugation of disease and to the building of a free human society. As a citizen it is his duty to make responsible use of this power, contributing:

[6] *Aequanimitas, With other Addresses to Medical Students, Nurses and Practitioners of Medicine*, "Unity, Peace, and Concord," Sir William Osler, P. Blakiston's Son & Co., Inc., Philadelphia, 1932, p. 428.

[7] Ibid. "Chauvinism in Medicine," p. 267.

Humanistically, to the relief of needless suffering and the postponement of death until the appointed time;

Scientifically, to the maturation of the full world potential in research;

Economically, to the building of man to his full productive capacity;

Propaganda-wise—if you will, to the infusion of the concepts and techniques of freedom among the rising masses of the world; and

Politically, to the erection of bridges of friendship and trust among men everywhere.

These are noble goals for us to seek, a proper use of our powers.

We are, within limits, using our power. Never in medical history has there been anything to equal the scope and range of today's activity in health on the world stage. In the scientific area, international congresses and conferences cover the whole range of technical fields. Students and research workers are flowing across national boundaries in unprecedented numbers. Thousands of unheralded missionary physicians are carrying on their traditional work. Within the past weeks, over 2,000 American physicians, responding to the appeals of Tom Dooley, have volunteered significant periods of their time for work in primitive areas, with MEDICO.

However, it is in the area of organized inter-governmental action that the greatest expansion has occurred. At the beginning of the century there was no official inter-governmental health activity, no international health organization. This week a routine report from the Department of State to the Congress, discusses the health work of 14 international organizations of which the United States is a member. These range from the World Health Organization, the United Nations Children's Fund, the International Atomic Energy Agency to the Caribbean Commission and the South Pacific Commission.

The World Health Organization is the central structure in this complex. Created following World War II, it now includes 90 Member Nations and has a staff of 1,900 people working in its Geneva headquarters, its 6 regional offices in Copenhagen, Alexandria, Brazzaville, New Delhi, Manila, and Washington, and in 800 projects in 124 countries and territories. The annual World Health Assembly, at which its policies are determined and budget established, provides for the first time in history a regular recurrent forum in which the world health authorities can meet to exchange views and develop common programs for the general good.

The supporting structure of the World Health Organization includes some 38 panels composed of more than 1,400 experts in the wide range of medical and health sciences. From these panels are drawn, according to the needs at any time, expert committees to study special problems. In the 12 years of the life of the Organization, 120 such committees have met and rendered authoritative reports on a wide range of subjects.

The projects through which the Organization assisted health administration in 1959 included 59 in malaria in 55 countries; 24 in yaws; 16 in leprosy; and 51 in tuberculosis. They included also 30 projects designed to help in the development of local and rural health services; 16 in occupational health; 23 in health education; 44 in maternal and child health; 70 in nursing; and 55 in environmental sanitation.

During 1959 the Organization awarded 1,431 fellowships. Of these 56 per cent went to physicians, 12 per cent to nurses and midwives, 7 per cent to sanitarians, and 25 per cent to other health personnel. More than 10,000 fellowships have been awarded by WHO.

Additionally, it has developed international quarantine regulations to which 170 countries or territories now subscribe, totally or in part. It broadcasts daily reports on a world network giving the incidence of pestilential diseases in every corner of the world. It has developed an international pharmacopoea, established official generic names for over 500 drugs in common use and developed internationally accepted standards for over 100 drugs and biologicals.

Last year WHO issued the *First Report on the World Health Situation*, a volume in which the health problems, resources and facilities of 157 countries and territories are analyzed. Presently, it is embarking on an intensified program for the stimulation and coordination of research, under the guidance of a Research Advisory Council composed of world leaders in medical research.

The most dramatic WHO program is that aimed at the total eradication of malaria. Here, for the first time, the nations of the world have pledged themselves to an all-out battle to conquer a disease. The United States has made special contributions to the WHO and the PAHO totaling $19.5 million for this campaign, and has given additional support through other agencies totaling some $50,000,000.

These figures perhaps give some indication of the scope and nature of the work being carried on by this new agency.

This, however, is not the limit of activity. The United States is carrying on an extensive technical assistance program in health under the International Cooperation Administration of the Department of State, which has health missions in over 40 countries. This program is under the direction of my distinguished classmate, Dr. Eugene Campbell. Its goals in the countries in which it is operating are identical with those of WHO—the control of mass preventable disease and the building of strong, competent national and local health services. The Public Health Service which is giving active day-in-and-day-out support to these programs, is also investing some four to five million dollars through the National Institutes

of Health annually in the support of medical research and training of research workers in foreign countries.

It is revealing to note the fact that the United States which before World War II was contributing only some 40 thousand dollars to international health work, expects this year to contribute one hundred million dollars to this work, through the various official programs of the international organizations and of our own Government agencies.

Nor is this yet the end. Through the Colombo Plan the nations of the British Commonwealth are providing mutual assistance to each other. The United Kingdom, Canada and Australia and the other highly developed members of the Commonwealth are assisting India, Ceylon and other less highly developed members. New Zealand, for instance, has contributed one million pounds to the new All India Institute of Medicine in New Delhi; Canada, $500,000 worth of cobalt therapy equipment to India; and Australia has sent 21 medical personnel to Malay.

The Soviet Union is also giving direct aid in health, providing technical assistance, for instance, in the control of smallpox in Iraq and making a major contribution of over $20,000,000 for the development of drug production in India.

Medicine is truly on the march on the world scene. You will say quite correctly that all this is not being done by physicians alone. Sanitation, the application of insecticides, and the manufacture of drugs are not primarily medical activities. The fact, however, is that the leadership and driving force behind these programs emanates from physicians.

The powers that I have mentioned are thus used to assist the underprivileged masses in attaining a better life.

This leads us back to consideration of the conflicts between the ideologies to which I have referred. Where do we stand in this? It is indeed a difficult question and one for which the answers are not readily at hand. I can only express certain beliefs that may be personal, but are widely shared. On the basis of over a decade of close relationships with Soviet medical leaders and medical scientists in their own country and on the international stage, I am convinced that our Soviet colleagues are motivated by Hippocratic ideals and I am convinced that their medical scientists are motivated by the same scientific drive, as well as, the same curiosity and interests that motivate our own medical scientists. Unfortunately, there exists a dichotomy between these interests and motivations and those at the political level. Our colleagues are at times used for the promotion of straight political goals. They are caught up in the realities of the world struggle, on the side of tyranny.

Recognition of the political overlay, however, should not, and has not,

deflected us from an attempt to develop a medical bridge between our two nations. Nor has it interfered with our continuing support of scientific goals that can be attained through working with our Soviet colleagues. Last month the United States concluded an agreement which provides for an expansion of cooperation between American and Soviet scientists. Under it the Ministry of Health will send to the United States, during the next two years, for short visits, missions to study our research in the fields of cancer, cardiovascular disease, poliomyelitis, thoracic surgery, genetics of viruses and industrial medicine and hygiene. The Public Health Service will send to Russia similar missions composed of Governmental and non-Governmental scientists to study infectious diseases; neuro-physiology and pathology; metabolism and genetics; medical ecology; and maternal and child health and related research, particularly peri-natal research. The agreement provides further for annual conferences of American and Soviet scientists of the Soviet Institute of Experimental and Clinical Oncology and, on the American side, the Sloan-Kettering Institute and the National Cancer Institute; in the field of cardiovascular diseases, between the scientists of the Soviet Institute of Therapy, and our National Heart Institute and other institutes in both countries; in the field of poliomyelitis between the Soviet Institute for the Study of Poliomyelitis and the U. S. National Institutes of Health and the Research Foundation of the Children's Hospital in Cincinnati, among other interested institutes.

It is planned that these meetings will lead to the exchange of top-level scientists and bench-level workers in these and perhaps other fields. It is hoped, and expected, that these exchanges will contribute to the lessening of tensions, through the creation of constructive and friendly relationships between scientific colleagues of the two nations.

In the meantime, we fully expect to continue to cooperate with the Soviets in the WHO. Perhaps together we can help the masses of mankind toward the attainment of the better life they are anxiously anticipating and so impatient to attain. It must be our constant purpose, of course, to do this in such a way that the people of other countries attain their goals themselves under a system of their choice, that leaves them free to run their affairs and govern themselves as they see fit.

The glaring contrast between the underdeveloped countries, such as Afghanistan, India and Pakistan, and their neighbors living under tyranny must be wiped out before the glitter of progress blinds the masses of these areas to the tragedy of tyranny.

We have not answered the questions raised in our mind by Thucydides. But we have found, I hope, that as physicians we have the great power to lessen subjugation by disease. We have seen that we are doing more than ever in history toward this goal, but, noted also, that we have failed in

58 years to attain the goals glimpsed by Osler at the turn of the century. In regard to the major conflict of our times we have noted that we are utilizing our powers to bridge gaps and to build a better life for the underprivileged. We hope that in this we are contributing to the lessening of world tension and the strengthening of the base of freedom among hundreds of millions of our fellow men.

We are left with the question, are we using our powers sufficiently, wisely and fully to meet the urgent requirements of our times? This question I cannot answer, but leave with you.

Let me in closing, quote Dr. Osler—for the last time today—he said in Montreal in 1902: "There seems to be no limit to the possibilities of scientific medicine, and while philanthropists are turning to it as the hope of humanity, philosophers see, as in some far-off vision, a science from which may come in the prophetic words of the Son of Sirach, 'Peace over all the earth.' "[8]

[8] Ibid. "Chauvinism in Medicine," p. 269.

KAPOSI'S SARCOMA IN A FATHER AND SON

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Though Kaposi's sarcoma is recognized to occur particularly in individuals born in Russia, Poland and Italy, and is thought by many to appear especially in those of Italian or Jewish ethnic origin, its appearance in members of a single family is rare. In his monograph, Bluefarb (1) noted only one instance of the disease affecting several members of a family. This was Greco's report of three brothers and a son of one of the brothers with Kaposi's sarcoma (2).

The following is a case report of a father and son with this disease.

CASE REPORTS

Case 1. (JHH №397629): A white, 73 year old man, born in Roumania of Jewish ethnic origin, was seen at the Johns Hopkins Out-Patient Department September 19, 1949, because of reddish growths on his right thigh. The asymptomatic lesions had been present for three months.

Upon examination, three dark red, smooth nodules were noted to be present on the posterior surface of the middle third of the right thigh, three similar nodules on the right heel, and one on the medial surface of the right ankle. A 3 centimeter non-tender, normal colored lymph node was palpable in the right inguinal region. A clinical impression of Kaposi's sarcoma was made and was confirmed by microscopic examination of a biopsy specimen of a skin lesion of the right thigh. New lesions appeared during the next year so that examination on May 11, 1950 disclosed an additional lesion on the anterior and another on the lateral surface of the right thigh, as well as a 0.8 centimeter papule on the right popliteal area.

Four days prior to his final hospital admission on May 19, 1951, he complained of malaise, insomnia and anorexia. This was followed by marked hyperpyrexia, coma and expiration on May 23, 1951. Postmortem study showed death to have been caused by diffuse interstitial myocarditis. Among other autopsy findings was Kaposi's sarcoma of the skin of the right foot, ankle, leg and thigh and of the right pelvic lymph nodes and the cortex of the right adrenal gland.

Microscopic examination of a biopsy specimen of one of the lesions of the skin of the right thigh showed a fairly well circumscribed but not encapsulated extensive tumor made up for the most part of many hematoxylin-staining, spindle-shaped cells lying in strands which extended in many directions irregularly. The nuclei was round, oval and irregular with differences in staining qualities. Only a few mitotic figures were present. In addition, there were numerous blood vessels, some of which exhibited endothelial proliferation. Some free erythrocytes were present in the edematous stroma. Interspersed with the spindle cells and blood vessels were many small and large mononuclear cells and plasma cells (Fig. 1).

Microscopic examination of an autopsy specimen of an involved pelvic lymph node

showed marked disorganization of the normal architecture because of many groups of loosely arranged hematoxylin-staining spindle-shaped cells lying in strands which extended irregularly in many directions. The nuclei were irregular in shape and staining, but only a few mitotic figures were noted. Both within and without the aggregates of spindle-shaped cells were many plasma cells as well as small and large mononuclear cells (Fig. 2).

Microscopic examination of an autopsy specimen of the right adrenal cortex showed a small area of invasion by loosely arranged hematoxylin-staining spindle cells lying in strands and extending in various directions irregularly. The nuclei of the spindle-shaped cells varied as to shape and staining quality. Interspersed between and particularly surrounding the spindle cells were individual and grouped inflammatory cells, mostly plasma cells (Fig. 3).

Case 2: A 64 year old white man, son of Case 1, was seen June 23, 1959 complaining of the presence of several asymptomatic bluish areas on the left foot. He had first noted these only two days previously. Except for allergic conjunctivitis, bronchial asthma and a bilateral inguinal herniorrhaphy, the past history was essentially negative.

A 0.8 centimeter purple smooth firm semi-globular nodule was noted on the lateral surface of the left foot inferior to the lateral malleolus. Anterior to this nodule were three similar lesions, none larger than 0.4 centimeter in diameter. Another similar 0.5 centimeter papule was present on the anterior surface of the same foot. His general physical examination and hemogram were normal (Fig. 4).

Microscopic examination of a biopsy specimen of a papule on the lateral surface of the left foot confirmed the clinical impression of Kaposi's sarcoma. The essential pathology was in the mid-dermis where a large mass of proliferating fibrous, vascular and inflammatory elements was present. Many blood vessels were seen at random. In some areas red blood cells were free in the stroma. Spindle cells were arranged in

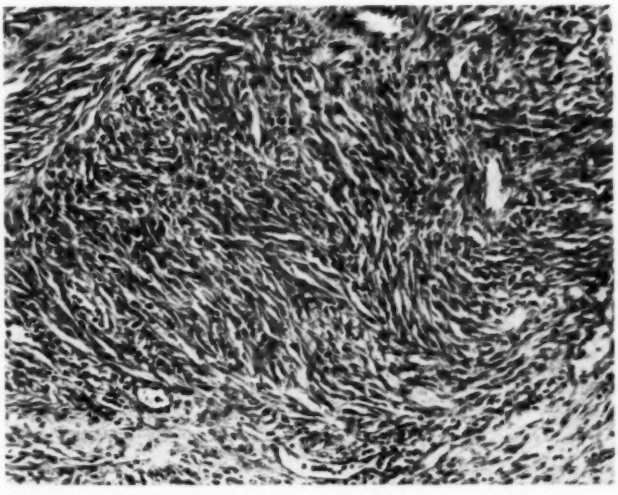

FIG. 1. (Case 1) (× 200)—Spindle cells, new blood vessels and inflammatory cells in skin nodule right thigh.

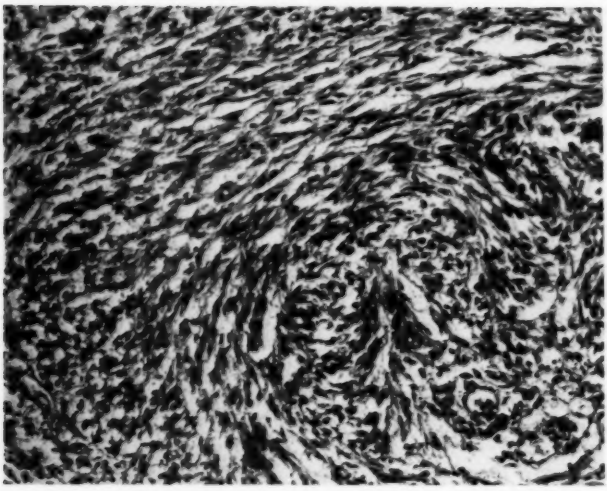

FIG. 2. (Case 1) (× 200)—Invasion of spindle cells and inflammatory cells in right pelvic lymph node.

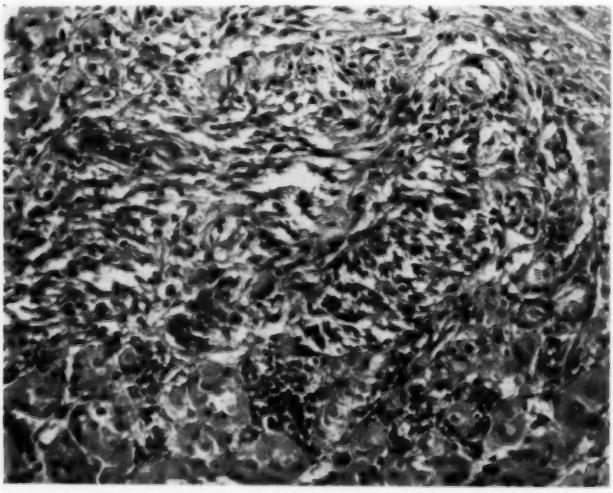

FIG. 3. (Case 1) (× 200)—Invasion of spindle cells and inflammatory infiltrate in right adrenal cortex.

strands and in irregular masses, with few nuclei exhibiting increased mitoses. Many plasma cells, some mononuclear cells, and a few eosinophiles were present among the spindle cells and blood vessels (Fig. 5).

Twelve x-ray treatments at weekly intervals, 75 roentgens each, (factors 60 KV, 10

FIG. 4. (Case 2)—Nodules and papules on lateral surface right ankle.

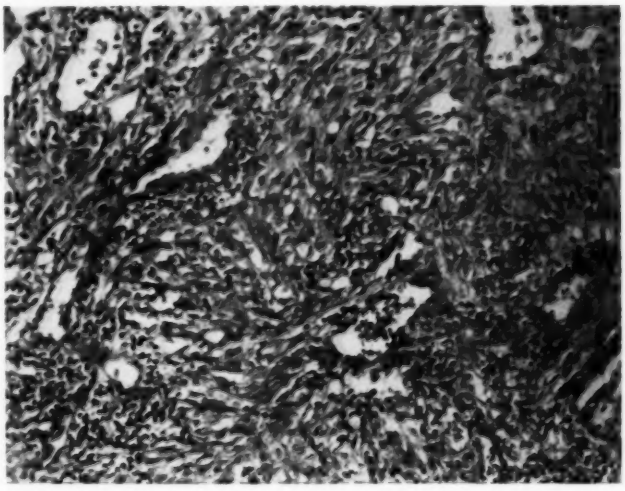

FIG. 5. (Case 2) (× 200)—New blood vessels, spindle cells and inflammatory infiltrate in skin papule of left ankle.

MA, 10 inch STD, no filtration, HVL 0.45 mm.Al) were administered. The lesions gradually lightened in color and became flatter so that two months after the completion of radiation therapy, none of the lesions was visible or palpable. The patient is continuing under observation.

SUMMARY

Kaposi's sarcoma is reported in a father and a son. Postmortem study showed that the father had involvement not only of the skin, but also of the pelvic lymph nodes and the cortex of an adrenal gland. The son, as far as can be ascertained clinically, has involvement only of the skin.

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SERUM CHOLESTEROL VALUES IN A HOSPITAL POPULATION

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The study reported here is a survey of serum cholesterol determinations performed in the routine laboratory of a large general hospital. It was undertaken for the following purposes:

1) To determine, in approximate terms, the range of the serum cholesterol level in persons without clinical evidence of disease known to influence the level.

2) To compare the mean serum cholesterol values in patients with various forms of "atheromatous disease" with those obtained in others without evidence of such disease.

MATERIAL AND METHODS

The material for this study consisted of all cholesterol determinations recorded in the Clinical Chemistry Laboratory at the Johns Hopkins Hospital during 1958. The determinations were performed by Dr. Mary V. Buell's adaptation of the Sackett (1) modification of the Bloor method. Details of the method and its reproducibility have been published by Thomas (2). The standard error of 11.2 mg. per 100 ml. compares favorably with any from the five collaborating laboratories in a cooperative study reported in 1958 (3). Since the diurnal variation of serum cholesterol is negligible (4, 5), no attention was paid to the time at which the blood was drawn; most, however, were fasting specimens. The method gives higher values than does the Sperry-Schoenheimer method.

The clinical record of each patient was reviewed and, in addition to serum cholesterol determination, the following information recorded on Royal-McBee punch cards: name, age, sex, race, date of cholesterol determination and diagnoses according to the criteria indicated below. Children under 14 years of age were excluded from study. Information relative to five special categories was recorded so that individuals with certain conditions known to influence serum cholesterol could be excluded in the final analysis of the level in relation to atheroma. These five conditions are cited below with the criteria used:

1. *Diabetes mellitus.* A clinical diagnosis of this condition was sufficient.
2. *Thyroid disease.* Any patient who had demonstrable disorder of function of the thyroid gland, who had a history of such disorder, or who was taking thyroid extract was removed from the series.
3. *Liver disease.* This group included any patient with hepatitis, cirrhosis, "fatty liver" or a serum bilirubin above 2.0 mg. per cent.
4. *Kidney disease.* The criteria included a diagnosis of nephritis or nephrosis, or a blood urea above 45 mg. per cent without evident extrarenal cause.
5. *Pregnancy.* Pregnant women were excluded since the lipids tend to rise during pregnancy (6).

[1] During conduct of this study, Dr. Manley held a research fellowship from the National Heart Institute.

On the punch-card, a diagnostic entry was made for the following "atheromatous" conditions[2] according to the following criteria:

1. *Cerebrovascular disease:* Cerebral hemorrhage, cerebral thrombosis, cerebral arteriosclerosis. Where these conditions were associated with neoplasm, inflammatory disease, surgical intervention or trauma, they were not recorded under "atheroma."
2. *Coronary artery disease:* Angina pectoris, coronary insufficiency, myocardial infarction and arteriosclerotic heart disease. A further subdivision of this group was made as follows:
   A. *Acute myocardial infarction.* In this group was recorded any patient diagnosed as having had a myocardial infarction within the two months prior to the cholesterol estimation.
   B. *Chronic coronary artery disease.* All patients with a diagnosis of angina pectoris, coronary insufficiency, or a cardiac infarction more than two months prior to the cholesterol estimation were so designated.
   C. *Congestive cardiac failure.* Patients suffering from cardiac failure due to forms of heart disease other than arteriosclerotic were excluded.
3. *Peripheral vascular disease:* This group included patients with obliterative vascular disease; persons with symptoms due to emboli, trauma or infection were excluded.
4. *Hypertension:* Patients with systolic pressure above 160 mm. Hg or diastolic pressure above 100 mm. Hg, or both, were included.

In order that in the final analysis something could be known about the large group of patients without atheromatous disease with which the various subdivisions of atheroma were being compared, all diagnoses were noted at the original survey of the clinical records and indicated on the punch card. In the card-punching, non-atheromatous diseases were assigned to various categories such as Acute Infections, Chronic Infections, Neoplasms, etc. Originally 21 categories were made but this proved to be too cumbersome and some diseases were finally selected because of their possible effect on the serum cholesterol or because these patients seemed to be otherwise normal. The categories are listed below:

1. *Surgery*—Patients admitted for gynecological, genitourinary, orthopedic or otolaryngologic surgery, hernia repair or removal of benign tumors.
2. *Neoplasms*—Any malignant condition, including leukemia.
3. *Malabsorption*—Ulcerative colitis, sprue, regional ileitis, post-gastrectomy syndromes.
4. *Obesity*, in the judgment of the physician.
5. *Stress*—Any patient diagnosed as having anxiety neurosis, tension state, spastic colon, migraine or allied conditions. Frank mental disorders were not included.
6. *Gastrointestinal*—"Peptic" ulcer, diverticulum, hiatus hernia.
7. *Miscellaneous*—Sarcoidosis, latent syphilis, Marfan's syndrome, Tietze's disease, etc.
8. *Normal*—A certain number of patients came to the hospital for a routine check-up and were found to be clinically normal.

When repeated determinations of serum cholesterol were made in the same patient, only the first in 1958 was used in this analysis. Storage of the punch cards in alphabetical

[2] The word 'atheromatous' will be employed as a convenient generic term for the cardiovascular diseases under scrutiny, although striking specific manifestations of atheroma may not have been present in all of them; e.g., many individuals in the sub-category "hypertension."

order by the patient's name aided identification of duplicate readings. After punching, all cards were checked once for errors. The cards were sorted manually by the ice-pick method and the cards were counted by a Pitney-Bowes Tickometer.

In the statistical analysis, the differences between means were tested by Student's *t*-test. When sample size was less than 30 or when the variances differed by more than 50 per cent, the degrees of freedom for finding the *t*-value were computed by Welch's method (7):

$$\text{d.f.} = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\frac{S_1^4}{n_1^2(n_1 - 1)} + \frac{S_2^4}{n_2^2(n_2 - 1)}}$$

where S_1^2 = variance of first sample,
n_1 = number in first sample,
S_2^2 = variance of second sample,
n_2 = number in second sample.

RESULTS

During 1958, at least one serum cholesterol estimation was performed in 4649 patients over the age of 14 years. From the hospital charts or private physicians, information was obtained on 4074 of these, or 87 per cent. To determine whether the records found and those not found represent similar groups, the frequency distributions of the serum cholesterol levels were compared. Figure 1 demonstrates that there was no striking difference

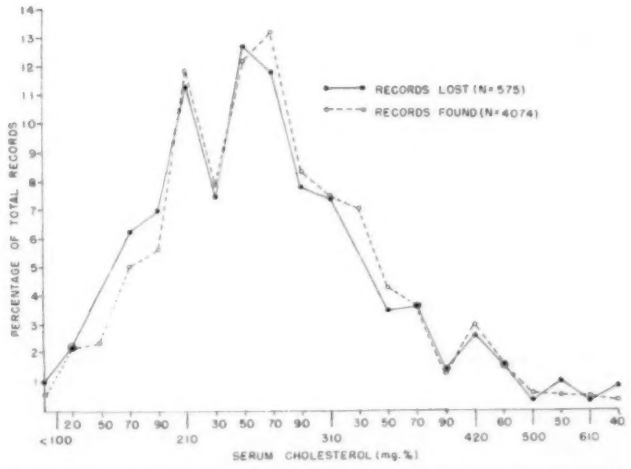


FIG. 1. A comparison of the frequency distribution of serum cholesterol levels in patients whose records were found for review and in those whose records were lost. The differences are not considered significant.

with regard to serum cholesterol between the 575 cases in which the chart could not be traced and the remainder.

Table I indicates those persons remaining after removal of cases because of diabetes or pregnancy, or disease of the thyroid, liver or kidneys—a total of 2,999 persons. These were first grouped according to sex and race, and then divided into 20-year age-groups.

Figure 2A–H shows cumulative frequency distributions for the 4 sex and race groups of all cases and the 20 to 39 and 40 to 59 age classes are compared. Although numbers are relatively small, the curves provide a basis for assessing the significance of a given cholesterol value determined under the conditions of this study. For example, a serum cholesterol value of 400 mg. per cent in a white male between 40 and 59 years of age falls in the high 5 percentile and can be construed as "abnormal."

TABLE I

Serum Cholesterol

All Cases

(Minus diabetes, pregnancy, and thyroid, liver, and kidney diseases)

N = 2999

	Age Range	n	$\bar{x}$	S^2	S.D.	S.E. of $\bar{x}$
W. M.	14–19	24	224	2148	46.3	10.3
	20–39	183	252.2	4046	63.5	4.7
	40–59	693	277.5	4564	67.5	2.6
	60–70+	368	258.2	4875	69.8	3.6
	Total	1268	267.3	4669	68.3	1.9
W. F.	14–19	32	219.7	3784	61.5	10.9
	20–39	207	239.3	3617	60.1	4.2
	40–59	509	290.6	5474	73.9	3.1
	60–70+	295	290.2	6430	80.1	4.7
	Total	1043	278.1	5833	76.4	2.4
C. M.	14–19	6	213	3100	55.0	22.9
	20–39	51	211.2	3588	59.9	8.4
	40–59	135	235.9	4051	63.6	5.5
	60–70+	79	246.3	6711	81.9	9.2
	Total	271	233.8	4850	69.6	4.2
C. F.	14–19	10	176	2111	46	14.8
	20–39	111	223.9	1848	42.9	4.1
	40–59	182	267.9	5352	73.1	5.4
	60–70+	114	277	7708	87.7	8.3
	Total	417	256.5	5774	75.9	3.7

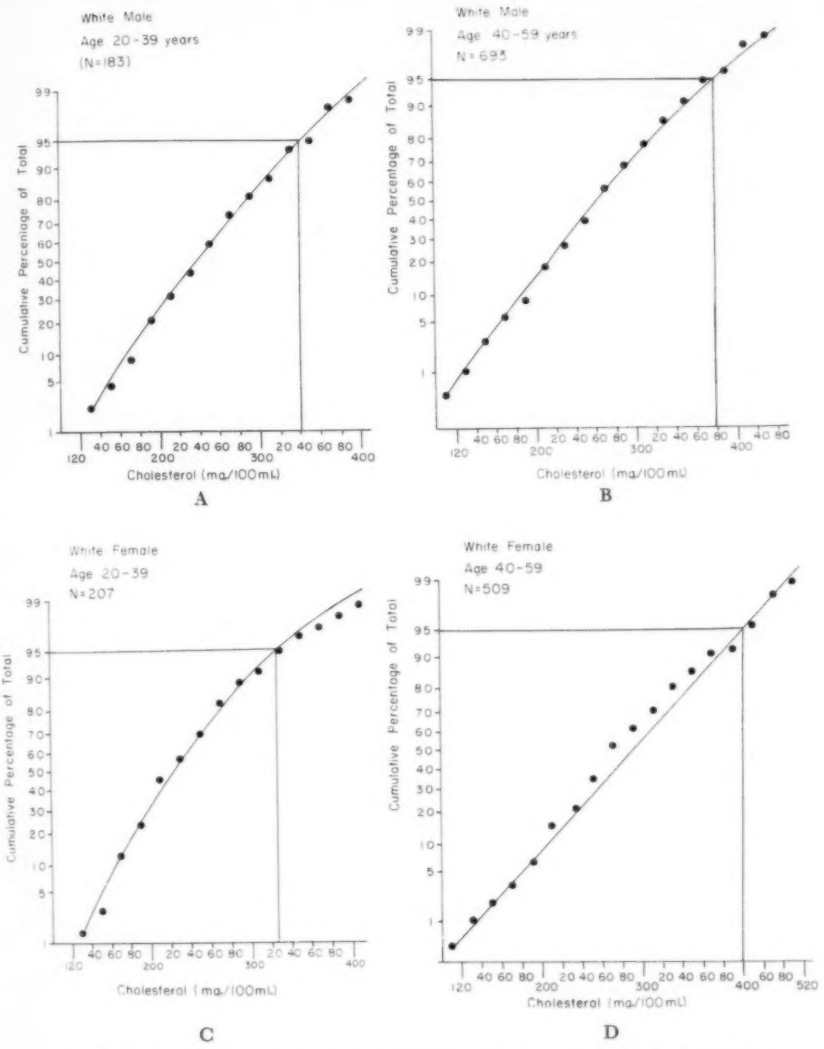


FIG. 2, A–H. Cumulative frequency distributions for serum cholesterol levels in two age groups for each of the two races and sexes. In each case the 95 per cent level is indicated. All cases (minus diabetes, pregnancy and thyroid, liver and kidney disease) are included, regardless of whether "atheromatous" disease was present or not.

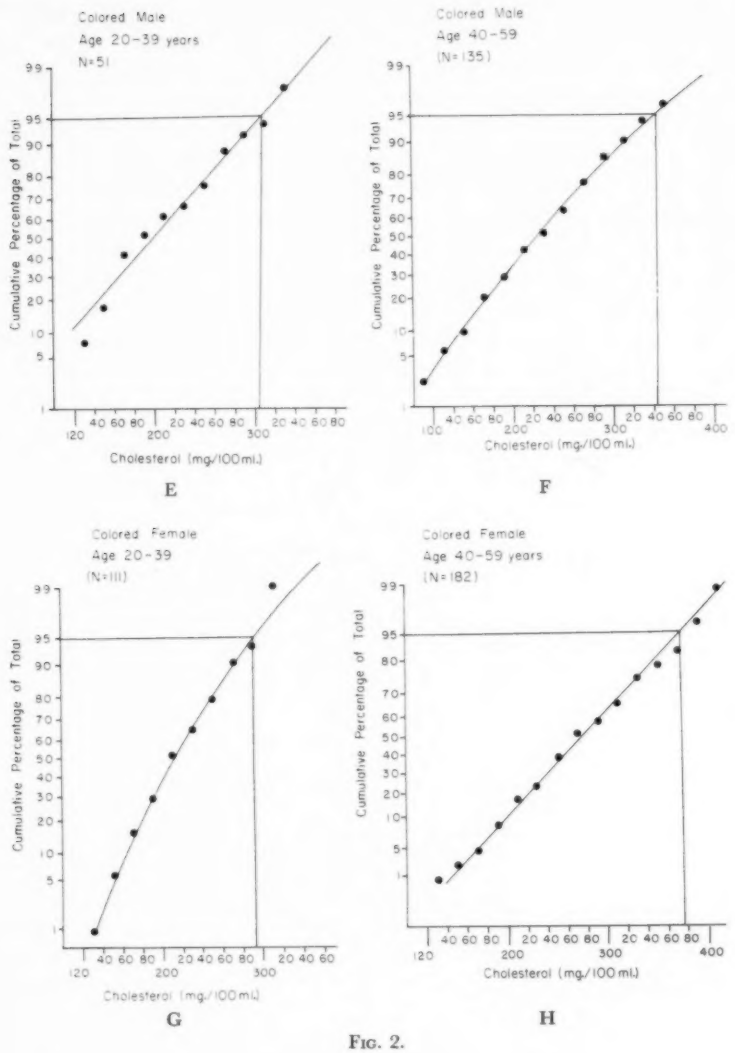


FIG. 2.

In Table II the atheromatous diseases are compared with the rest. Several points emerge:

1. In the non-atheroma group the mean cholesterol rises appreciably with age in all groups except the colored males where the rise is negligible.

2. The difference between W.M. and W.F. at age 40 to 59 is significant ($p < .05$), whereas at ages 20 to 39, W.M. had tended to be higher but not

TABLE II

Serum Cholesterol

All Cases

(Minus diabetes, pregnancy, and thyroid, liver, and kidney diseases)

N = 2999

	Age Range	Non Atheroma					Atheroma					
		n	$\bar{x}$	S^2	S.D.	S.E. of $\bar{x}$	n	$\bar{x}$	S^2	S.D.	S.E. of $\bar{x}$	
W. M.	14–19	24	224	2148	46.3	10.3						
	20–39	153	243.8	3252	57	4.6	30	297.0	5827	76	13.8	**
	40–59	416	268.4	3954	63	3.08	277	291.3	5181	72	4.3	**
	60–70+	140	258.8	5066	72	6.1	228	257.8	4779	69	4.6	
	Total	733	259.9	4078	64	2.3	535	277.4	5312	73	3.1	**
W. F.	14–19	31	217.4	3740	61	10.8	1	290				
	20–39	193	238.9	3684	61	4.4	9	247.7	2350	48	16	
	40–59	363	279.9	4358	66	3.4	146	317.2	7300	85	7.1	**
	60–70+	121	284.9	6233	79	7.2	174	296.8	6571	81	6.1	
	Total	713	266.7	4909	70	2.6	330	302.9	6947	83	4.6	**
C. M.	14–19	6	213.3	3100	55	22.9						
	20–39	43	204.1	2666	52	8.0	8	248.7	7671	87.5	31.2	
	40–59	77	215.2	3459	59	6.8	58	263.4	3553	60	7.9	***
	60–70+	28	218.9	2470	50	9.4	51	261.4	8284	91	12.8	**
	Total	154	212.7	3131	56	4.5	117	261.5	5793	76	7.1	***
C. F.	14–19	10	176.0	2111	46	14.8						
	20–39	88	220.1	1732	42	4.4	23	238.7	2118	46	9.5	
	40–59	85	241.5	4530	67	7.2	97	291.0	4970	70	7.1	***
	60–70+	40	250.5	7015	84	13.3	74	291.3	7588	87	10.1	*
	Total	223	231.7	4004	63	4.2	194	284.9	5870	77	5.5	***

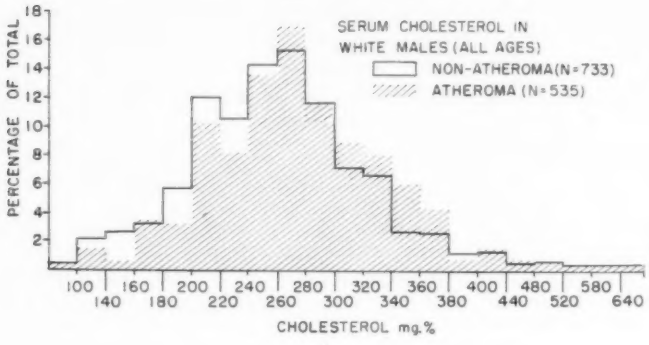
* $p < .05 > .01$ ** $p < .01$ *** $p < .001$.

Levels of significance refer to differences between atheroma and non-atheroma groups.

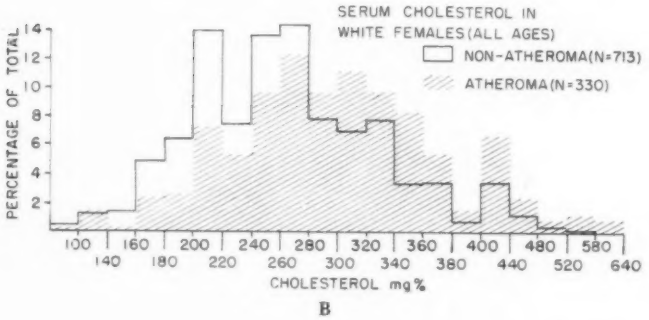
significantly so. This accords with the findings of various workers (8, 9) that in white males the serum cholesterol tends to rise from adolescence to middle life, whereas in white females the level does not begin to increase until middle life.

3. The comparison of the atheroma and non-atheroma groups shows that, on the whole, the former tends to have a higher mean serum cholesterol (Fig. 3A–D). As will be seen later, the differences become more marked when the atheroma group is subdivided.

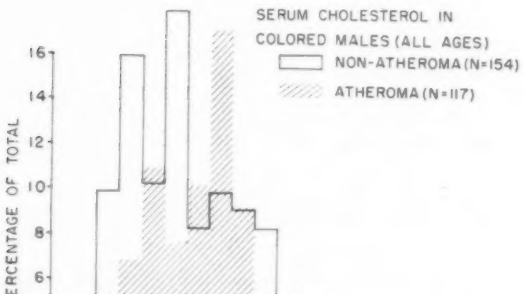
More detailed consideration of "atheroma". Peripheral arteriosclerosis,



A



B



C

FIG. 3, A–D. A comparison of the serum cholesterol distribution in "atheroma" and "non-atheroma."

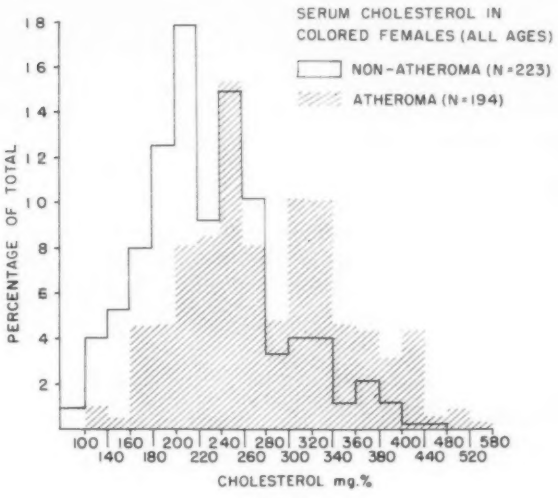


FIG. 3D.

cerebral arteriosclerosis and coronary artery disease may be relatively independent; each may exist in the absence of evidence of the others (10, 11). It was for this reason that the atheroma group was subdivided (Table III). Coronary artery disease was separated into "acute" and "chronic," since a fall in blood lipids occurs following myocardial infarction and several weeks may elapse before the pattern returns to that level present before infarction (12). A separated group was made for patients with congestive cardiac failure since it was noticed during collection of these data that such patients appeared to have lower cholesterol levels. The remaining patients, comprising hypertension, peripheral vascular disease, cerebrovascular disease and "arteriosclerotic heart disease," will be considered together and referred to hereafter as the "residual" group. Each subdivision contained patients of all ages. Table III shows the age distributions, as well as the means and variances for serum cholesterol in the several groups.

Patients were selected for these various groups because of the *main* atherosclerotic manifestation, e.g., infarction, angina, etc. A certain number of these were also suffering from hypertension, peripheral vascular disease, etc. No allowance was made for the latter factor, since the objective was a comparison of cholesterol level in those with one of the clear-cut manifestations, with that in the "residual" group.

The following points in the data on *white males* are of interest:

a. The highest mean level occurred in chronic coronary artery disease and this differed significantly ($p < .01$) from the "residual" group, and from

TABLE III

Serum Cholesterol in Sub-Groups of "Atheroma"

Sub-Group	n	$\bar{x}$	S^2	S.D.	S.E. of $\bar{x}$	Age Distribution						
						14–19	20–29	30–39	40–49	50–59	60–69	70+
W.M.												
Coronary artery disease												
Chronic	161	293.9	4182	64.7	5.1		1	8	35	62	44	11
Acute	71	280.1	3396	58.3	6.9			6	13	27	17	8
** Congestive cardiac failure	30	233.0	4317	65.7	11.9			1	3	4	11	11
** Residual	271	272.1	4680	68.4	4.2		2	12	36	97	77	49
W.F.												
Coronary artery disease												
Chronic	76	326.4	9601	97.9	11.2			2	16	19	29	10
Acute	14	342.1	6338	79.6	22.7				2	3	5	4
* Congestive cardiac failure	19	258.9	8011	89.5	20.8					4	8	7
** Residual	221	296.0	5604	74.8	5.1	1		7	28	74	71	40
C.M.												
Chronic	16	278.7	11426	106.6	27.3			1	3	5	4	3
Acute	16	261.2	14493	120.3	30.1			1	1	8	3	3
* Congestive cardiac failure	16	215.0	2000	44.7	11.2				1	5	3	7
*** Residual	70	264.6	4081	63.9	7.6		3	3	13	23	20	8
C.F.												
Chronic	13	296.9	6491	80.5	22.4				3	3	4	3
Acute	6	350	8280	90.9	39.5					1	2	3
Congestive cardiac failure	23	246.9	4841	69.6	14.8			3	1	9	6	4
* Residual	152	286.3	8111	90.0	7.3		5	15	33	47	34	18

The asterisks refer to the levels of significance of the difference indicated:
* $p < .05 > .01$ ** $p < .01$ *** $p < .001$.

the cardiac failure group ($p < .01$), but not from those with recent infarction.

b. The cardiac failure group was significantly ($p < .01$) below all other groups. Inspection of the age distribution showed that the majority (73.3 per cent) of those with cardiac failure were over 60 years of age, whereas the proportions of this age group in chronic coronary disease, acute infarction, and "residual" atheroma was 34.1, 35.2, and 46.5 per cent respectively. Since the cholesterol level appears to fall over the age of 60 years (Table II), the lower mean serum cholesterol level in congestive cardiac failure might be more apparent than real. However, the mean level for the cardiac failure group was significantly ($p < .05$) lower than that for the non-atheroma or atheroma group, aged 60 years or more. This suggests that the serum cholesterol is genuinely lowered in cardiac failure.

In the *white females* the trends were the same as for the white males, but the number of differences attaining significance were fewer. Thus, although the means appeared to differ widely the only significant disparities were between coronary artery disease (both recent and chronic) and the residual group ($p < .05$), and between the coronary artery group and congestive failure ($p < .01$).

The number of *colored males* was small. Again, while the trend is much the same as was seen in white males (the mean values in each section are lower), there was no significant difference demonstrable between coronary disease (acute and chronic) and the "residual" group. The mean for the congestive failure group was significantly lower than the other groups ($p < .01$).

In the *colored females*, due in part to the small numbers in the subdivisions of coronary artery disease, the only significant difference was that between the "residual" group and congestive cardiac failure ($p < .05$).

A higher proportion of colored females developed "atheromatous" disease before the age of 40, as compared with white females. All but two of these were included because of hypertension, which bears out the observation that hypertension develops at an earlier age in the colored race (13).

More detailed consideration of "non-atheroma". The make-up of the non-atheroma class was investigated by study of the several subclasses indicated in *Methods and Materials*. Since many persons had more than one disease, appreciable overlap exists between subclasses. Once cards for one subclass had been analyzed, they were returned to the stack and the next subclass analyzed. It is evident that an individual reading might appear in more than one category. For this reason a detailed analysis would not be warranted. The sole object was to obtain some impression of what was being used for comparison with atheroma.

In Table IV are seen the results of the analysis, together with the age distributions in the various categories. Those conditions between which a significant difference exists are joined by lines. No very striking differences exist except for neoplasia which tends to be accompanied by a low cholesterol in men. Obesity was associated with some elevation of serum cholesterol in white women. The other diseases not shown in Table IV were analyzed roughly and the distribution did not differ appreciably from those shown. It seemed, then, that with the possible exceptions of neoplasia and the malabsorption syndrome the nonatheromatous group was a reasonably homogeneous one as regards serum cholesterol level and, therefore, suitable for comparison with the atheromatous diseases.

In view of the apparent tendency for neoplasia to be associated with a lower cholesterol level, the atheromatous groups were checked for coin-

TABLE IV

Serum Cholesterol in Selected Sub-Groups of "Non-Atheroma"

Sub-Group	n	$\bar{x}$	S^2	S.D.	S.E. of $\bar{x}$	Age Distribution						
						14–19	20–29	30–39	40–49	50–59	60–69	70+
W.M.												
** Neoplasm	52	214.4	4538	67.7	9.4	1	3	1	9	17	15	6
** Malabsorption	17	197.0	2437	49.3	12.0	1	3	3	5	4	1	
Obesity	90	275.1	3736	61.5	6.5	2	3	13	26	34	12	
** "Stress"	124	274.9	3089	55.5	5.1	1	8	33	37	33	9	3
"Normal"	60	265.3	2597	50.9	6.6	2	3	13	22	15	4	1
W.F.												
Neoplasm	35	230.6	3488	59.1	10.1		3		7	13	8	4
Malabsorption	26	233.1	5720	75.5	14.8	2	2	10	9	1	1	1
Obesity	87	297.5	5520	74.2	7.9	5	3	9	29	18	17	6
"Stress"	166	280.7	3382	58.1	4.5	7	8	31	52	41	23	4
"Normal"	35	261.1	6276	79.2	13.4	3	8	6	9	7	2	
C.M.												
Neoplasm	18	191.6	3500	59.1	14.1			1	6	6	5	
Gastrointestinal	21	210.5	2105	45.8	9.9		2	2	5	7	3	2
Malabsorption	10	220.0	6489	80.5	25.9		1		5	1	2	1
Miscellaneous	14	223.6	2331	48.2	13.1	2	1	2	6	2	1	
C.F.												
Neoplasm	15	244.7	5071	71.2	18.2		1	1	3	1	7	2
Obesity	29	257.5	4322	65.7	12.2		5	8	10	2	4	
Stress	15	247.3	3021	54.9	14.1		2	6	4	3	1	
GYN etc.	26	208.1	7472	86.4	16.9	1	4	5	8	3	3	2
Miscellaneous	30	219.3	2227	47.1	8.7		8	6	4	7	4	1

** $p < .01$ for differences indicated.

cident neoplasia. In 14 white males and 11 white females it was found that both atheroma and neoplasm were present. The mean for the white males was 230.7 mg. ± 18.1, and for the white females 250 mg. ± 19.9. These proved to be significantly lower ($p < .05$) than the means for atheromatous patients over 60 years of age in their respective sex and race groups. (All the atheromatous patients with neoplasm fell into that age class.)

Socio-economic class. The mechanics of the present study did not permit an examination of serum cholesterol levels in subgroups according to socio-economic status (except, of course, insofar as race is an indicator of such status). Perusal of the clinical records created the impression that estimation of serum cholesterol, often as part of a routine check-up, was more often performed in patients on the private services than on others.

CONCLUSIONS

The data reported here were derived from a mixed sample of the Johns Hopkins Hospital patient population with an unknown proportional representation of the races, sexes, and socio-economic groups. Despite these limitations, the following conclusions seem justified:

1. The mean serum cholesterol level tends to rise with age. In men this rise appears to commence earlier than in women and in both sexes the level does not change greatly after 60 years.

2. The mean serum cholesterol level tends to be higher in white patients than in the colored.

3. The mean level is higher in those diseases associated with atheroma than in other diseases.

4. The elevation is most marked in those persons suffering from coronary artery disease. Although this difference is not statistically significant in the colored female group, it is likely that this is due to small sample size.

5. In all sex and race classes the serum cholesterol is lower in arteriosclerotic cardiovascular disease with congestive heart failure than in other clinical forms of atheromatous disease.

6. The presence of neoplasia is associated with a lower serum cholesterol level, even when atheroma is present.

The agreement of these findings with trends observed in other series, including population studies, suggests that the sample was fairly representative of the hospital population and that the latter is reasonably representative of the local population, at least with respect to the more serious aspects of atheroma. The hospital population has one advantage over most general population samples, namely, accurate diagnosis. All cases of coronary artery disease are well documented and the normal subjects have been found to be free of clinically evident disease by thorough investigation. Follow-up information will be sought on those persons free of obvious atheromatous manifestations who have high serum cholesterol values, to see whether clinical evidence of atheroma supervenes.

Although data of interest and significance are provided by the determinations in 2,999 individuals in which kidney, liver, or thyroid disease or diabetes was not the indication for the test, these are group data. It might be seriously questioned what significance with reference to atheromatous disease can be attached to a serum cholesterol determination in an individual patient and whether the expense of such determinations justifies the return in information.

In individual cases data such as those presented in Figure 2 can be used

in the way illustrated by the following hypothetical example: A 35 year old white male with chest pain but without clinical evidences of diabetes or of disease of the thyroid, liver or kidney has a serum cholesterol level, determined in this laboratory, of 400 mg. per 100 ml. Since this value falls well above the 95 per cent level for his sub-group (Fig. 3A), the serum cholesterol level can be construed as abnormal and weighed, together with the other clinical information, in arriving at a diagnosis of angina pectoris.

SUMMARY

The serum cholesterol determinations in the Johns Hopkins Hospital during the year 1958 were the basis for this study. The clinical features of 87 per cent of the adult patients subjected to this test were analyzed. The effects of age, sex and race were in accord with previous reports. Diseases of an atheromatous nature were associated with a higher serum cholesterol value, but the overlap with other diseases and with the normal was considerable. The effects of various manifestations of atheroma were separately inspected and the results discussed.

ACKNOWLEDGMENT

It is a pleasure to record our debt of gratitude to the following: To all physicians who gave details of their private patients; to Professor J. Cornfield, Dr. Helen Abbey and Dr. John Gart for statistical advice; to the Medical Records Department for finding the majority of the histories requested; to the staff and students of the Moore Clinic, who assisted in the collection, recording and sorting of data; to Mr. William Howe of Royal McBee Corporation; and to Messrs. Pitney-Bowes Inc. who made the Tickometer available for the final counting of the cards.

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QUANTITATIVE DETERMINATION OF PORPHYRIN UPTAKE BY TUMOR TISSUE FOLLOWING PARENTERAL ADMINISTRATION

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Despite a background of clinical and laboratory observations attesting to the propensity of exogenous porphyrin to concentrate in tumor tissue (1–9), there are no quantitative data regarding the phenomena. Such information would seem a necessary prerequisite for the investigation of this potentially valuable phenomenon. It is the purpose of this investigation to assess quantitatively artificial porphyrins in tumor and muscle after parenteral administration of hematoporphyrin in order to evaluate more accurately its selective uptake.

MATERIALS AND METHODS

Animals. Albino male Wistar rats weighing approximately 250 grams each were used in all experiments. Avascular pellets from about 1 cu. mm. of Walker carcinoma-sarcoma #256, obtained from the laboratory of Dr. George O. Gey, were implanted in the thigh of the rat through a #17 trochar. In some animals the tumor was implanted into the anterior chamber of the eye. The presence of the tumor in the anterior chamber of the eye permitted observation of the growth and necrotic changes of the tumor and of its red fluorescence after porphyrin administration. Porphyrin was given 11 days after tumor implantation and the animals sacrificed at different periods following this. The tissue specimens were carefully dissected to eliminate any necrotic areas and surface blood.

Porphyrins. Hematoporphyrin hydrochloride, NENCKI, recrystallized, obtained from the Cosmos International Corporation, New York, N. Y., was administered to the rats by intraperitoneal injections of solutions that contained 10 mg. per ml. in $\frac{1}{6}$ M. sodium lactate. The sample of hematoporphyrin hydrochloride used contained neither protoporphyrin nor deuteroporphyrin in an amount detectable by the extraction procedures used in this study.[3]

Separation of Porphyrins. The following separation scheme was adapted from that of Lemberg and Legge (10), and Schwartz, *et al.* (9).

1. Tissue was obtained as described below, weighed and minced finely. This tissue

[1] Henry Strong Denison Scholar for 1958–59.

[2] Supported in part by an institutional grant from the American Cancer Society, (IN-11).

[3] The absorption spectrum of this hematoporphyrin and the melting point of the dimethyl ester was determined in the laboratory of Dr. F. H. J. Figge of the University of Maryland School of Medicine and found in agreement with given values.

was homogenized in 15 ml. buffered acetic acid (4 parts glacial acetic acid to 1 part saturated sodium acetate).

2. The suspension was centrifuged at 4,000 r.p.m. for 5 minutes at 4°C. The supernatant was filtered into a suction flask through Whatman #50 filter paper. The pellet was homogenized in 15 ml. ethyl ether, and again centrifuged at 4,000 r.p.m. for 5 minutes. The resulting pellet was homogenized in 7.5 ml. ethyl ether, 7.5 ml. buffered acetic acid and the suspension filtered. All precipitated material was removed by filtration. The combined filtrate was made up to a total of 30 ml. of buffered acetic and 100 ml. ethyl ether.

3. The ether-acetic acid was washed in a separatory funnel three times with 20 ml. of 3 per cent sodium acetate and the ether phase saved. The washes were discarded after checking that there was no red fluorescence in the acetic acid-sodium acetate phase.

4. The ether was extracted six times with 5 ml. of 5 per cent HCl (by volume), or until there was no further red fluorescence in the HCl. The porphyrin content of the combined 5 per cent HCl extract was determined and designated "total porphyrin".

5. The 5 per cent HCl extract was brought to pH 5 with solid sodium acetate. The porphyrins were again taken up in ethyl ether by three 15 ml. extractions of the pH 5 solution.

6. The ether solution was extracted four times with ⅓ vol. 0.54 per cent HCl.

7. The ether solution from step 6 was extracted three times with ⅓ volume 8 per cent HCl or until there was no further red fluorescence in the extracts. The 8 per cent HCl was diluted to 5 per cent and the porphyrin content assayed and designated as the protoporphyrin fraction.

8. The 0.54 per cent HCl solution from step 6 was brought to 0.18 per cent HCl by partial neutralization with NaOH. This was extracted four times with ⅓ volume chloroform. Both the chloroform and the 0.18 per cent HCl solutions were saved.

9. The chloroform solution from step 8 was extracted four times with 5 per cent HCl or until no further red fluorescence appeared in the extracts. This sample was assayed and designated as the deuteroporphyrin fraction.

10. The 0.18 per cent HCl solution from step 8 was brought to 5 per cent HCl. This sample was assayed and designated as the hematoporphyrin fraction.

That this separation procedure adequately separated the porphyrins was shown by the disappearance of red fluorescence in successive extracts while a red fluorescence remained in the solution being extracted, for example, as in step 6. The term "fraction" is employed since the absolute purity of the substance in each assayed solution has not been unequivocally established. However, the spectra of these fractions are consistent with literature values of hemato-, proto-, and deuteroporphyrin respectively (10).

Quantitative Determination of Porphyrins. Porphyrin determinations were made by fluorometric and spectrophotometric techniques.

A. The porphyrin content of 5 per cent HCl solutions was determined by comparison of the red fluorescence under ultraviolet light with standards made up of hematoporphyrin. Differences in concentration could be detected within 2 micrograms per cent. The lower limit of fluorescence considered significant by this means was 10 micrograms hematoporphyrin per 100 ml.

The unit in which the concentration of the several porphyrins was expressed is micrograms porphyrin per gram tissue.

B. The spectrophotometric method utilized the absorption in the ultraviolet band, measuring each porphyrin at its peak wave length (14) in the Beckman DU Spectropho-

tometer. Hematoporphyrin fraction and deuteroporphyrin fraction were measured at 402 millimicra and protoporphyrin fraction at 408 millimicra. A straight line curve could be obtained using standard hematoporphyrin, and concentrations of 1.0 microgram porphyrin per 100 ml. could be accurately measured. The wave length of the peak absorption was the same when hematoporphyrin was dissolved in pure 5 per cent HCl and in 5 per cent HCl tissue extract (step 4, above) when the tissue contained negligible porphyrin.

RESULTS

Under ultraviolet light the red fluorescence of the tumor growing in the anterior chamber was first visible 4½ hours following the injection of 20 mg. hematoporphyrin intraperitoneally. It persisted for the five days that the last sacrificed animal lived. The tumor tissue also had a gross red fluorescence that was not present in surrounding muscle tissue. In the orbit an extremely brilliant red fluorescence was exhibited by the Harderian glands which are known to possess naturally a high concentration of protoporphyrin (11).

In Table I is summarized the data showing the uptake of porphyrin. Porphyrin was not present in muscle to a measurable extent. In tumor, it appeared in every animal that had received hematoporphyrin. The values for the concentration of porphyrin are expressed in adjacent columns according to the method of assay. The agreement between these values is close. Since each depends upon different properties of the porphyrin, the correlation supports the general accuracy of the assay techniques. When

TABLE I

Total Uptake of Porphyrin by Muscle and Tumor

Animal No.	Mg. hematoporphyrin injected	Hours of sacrifice after injection	Muscle		Tumor	
			Micrograms porphyrin per gm.		Micrograms porphyrin per gm.	
			Fluorometric	Spectrophotometric	Fluorometric	Spectrophotometric
1	0	11 days of tumor growth	0[1]	0[1]	0	0
2	0	13 days of tumor growth	0	0	0	0
3	20	8	0	0	2.68	2.63
4	20	8	0	0	2.25	2.54
5	20	24	0	0	1.84	1.43
6	20	24			2.81	2.97
7	40	24			6.61	10.22
8	80	24	0	0	6.02	8.95
9	20	48			4.25	4.96
10	20	120			1.98	2.30
11	20	120			2.24	1.65

[1] Less than 0.10 (microgram) per gram.

TABLE II

Content of Tumor Hemato-, Proto-, and Deuteroporphyrin

Animal No.	Micrograms Hematoporphyrin / gm. tumor		Micrograms Protoporphyrin / gm. tumor		Micrograms Deuteroporphyrin / gm. tumor	
	Fluorometric	Spectrophotometric	Fluorometric	Spectrophotometric	Fluorometric	Spectrophotometric
3	1.88	2.66	1.05	1.05	0.25	0.32
4	1.93	2.31	0.41	0.82	0.62	0.65
5	0.58	0.38	0.39	0.55	0.36	0.32
6	1.96	2.12	1.06	0.96	0.58	0.52
7	4.55	6.82	1.17	1.38	1.04	1.73
8	4.28	4.28	2.32	2.71	0.55	0.84
9	2.92	2.70	1.37	1.48	0.62	0.73
10	0.69	0.99	0.26	0.64	0.26	0.77
11	0.89	0.97	0.30	0.28	0.65	0.71

animal 7 was administered 40 mg. of hematoporphyrin more tumor porphyrin was found than in any of the animals who had received 20 mg. However, animal 8, which received 80 mg. of hematoporphyrin, showed no further increase in tumor porphyrin. It is not possible to determine the per cent of administered porphyrin that was taken up by the total tumor mass because not all tumor was obtained for analysis. It is estimated, however, to be far below 1 per cent of the porphyrin given.

Table II shows the results of separation of the porphyrins and quantitative analyses of each of the three porphyrin fractions. The values obtained by both assay techniques are again placed in adjacent columns and show good agreement. It can be seen that the major fraction in which the porphyrin content of the tumor is found regardless of the time between administration of hematoporphyrin and sacrifice of the animal is the hematoporphyrin fraction. In the animals sacrificed at 8 and 24 hours after hematoporphyrin administration, protoporphyrin fraction was found to be the next greatest and deuteroporphyrin fraction the least in all cases. The proportions of protoporphyrin fraction and deuteroporphyrin fraction in the animals sacrificed at 48 and 120 hours after hematoporphyrin administration do not show this same consistency.

DISCUSSION

These experiments clearly indicate that porphyrin administered to an animal in which a tumor is growing is concentrated in the tumor and not in control muscle tissue. Tissue other than muscle was not analyzed for porphyrin content either qualitatively or quantitatively. It was repeatedly observed on postmortem examination of the sacrificed rats that brilliant

red fluorescence under Wood's lamp occurred only in the tumor and the Harderian gland. If other tissues or organs contained more porphyrin than the control muscle, it was insufficient to confer apparent fluorescence. There is no ready explanation for this phenomenon as yet. It has been shown that rapidly growing tissues with a high mitotic index share this property (3). Evidence pointing towards tumor tissue possessing a unique affinity for porphyrin comes from the findings of Kosaki, *et al.* They described a phospholipid material, "malignolipin", obtained only from human tumor tissue, which has the property of specifically binding porphyrin (12).

These experiments also indicate that three different porphyrin fractions appeared in tumor after administration of only hematoporphyrin parentally. It cannot be established from these data whether hematoporphyrin was transformed to proto- and deuteroporphyrin in the tumor tissue or whether the transformation occurred prior to entry of the porphyrins into tumor. The former interpretation is suggested by the finding of Altman, *et al.* (13) that of the C14 incorporated into the carbinol carbon of the hydroxymethyl group of hematoporphyrin dimethyl ester incubated with bone marrow for 3 hours at 38°C. 5 per cent was found in protoporphyrin and 15 per cent was found in CO_2. If hematoporphyrin is transformed after its entry into the tumor tissue the transformations must be better understood so that radioactive labelling may be placed on the molecule and expected to remain there in high enough concentration to permit effective scintillation counting for diagnostic scanning.

SUMMARY

The uptake of systemically administered porphyrin by Walker carcinoma-sarcoma #256 transplanted to the anterior chamber and thigh has been quantitatively studied. It has been shown that the tumor concentrates porphyrin and that control muscle tissue does not. The finding of protoporphyrin and deuteroporphyin in the tumor tissue has been observed and quantitated. The significance of these studies with relation to porphyrin metabolism of tumor and the possible clinical application of the phenomenon have been briefly discussed.

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MULTIPLE MYELOMA WITH MYELOFIBROSIS AND WITH POLYCYTHEMIA VERA:

Further Evidence of a Relationship between the Myeloproliferative Disorders[1]

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The association between two or more of the diseases belonging to the group of myeloproliferative disorders has been reported frequently. It is well known that there is a close interrelation between myelofibrosis, myelocytic leukemia and polycythemia vera which on occasions makes separation into distinct entities difficult (1). In considering the relationships between these disorders, several authors (2, 3) have suggested that an unknown stimulus may react to initiate proliferative activity of primitive bone marrow cells. All of the bone marrow cells may be affected together or separately with distinct or transitional syndromes being produced. The purpose of this paper is to report two cases of multiple myeloma each associated with one of the disorders commonly included in the group of myeloproliferative diseases.

L. K., JHH #841511, a 58 year old white male, was admitted to the Johns Hopkins Hospital on April 1, 1959, because of weakness. Past history revealed that he had enjoyed good health until 3 years prior to admission when his local physician found that his red blood cell count was elevated. At that time he was in good general condition. He had a plethoric appearance and the conjunctivae were injected. There was no evidence of respiratory disease. The spleen was enlarged below the left costal margin.

The hematocrit value was 68 per cent; red blood cell count 7 million per cu. mm.; white blood cell count 9,000 per cu. mm. and a normal differential count. The serum uric acid was 10 mg. per 100 ml. Eight phlebotomies of 500 cc. each were performed in a period of several months and the patient improved markedly.

During the next two years 6 phlebotomies of 500 ml. each were necessary to maintain his hematocrit value in the normal range. In 1957 the white blood cell count was 14,800 per cu. mm. with 81 per cent neutrophils. The uric acid remained elevated. The patient remained quite well until 6 weeks prior to his first admission. At that time he developed an upper respiratory infection and was treated with a short course of Gantrisin®.

Thereafter his red blood cell count was discovered to be 4,100,000 per cu. mm. and the hematocrit value was 27 per cent. He was admitted to the hospital. He did not appear acutely ill but was quite pale. There was no icterus. There was a flame-shaped hemor-

[1] This investigation was supported in part by a research grant from The National Institutes of Health, United States Public Health Service.

rhage in the right fundus. There was marked sternal tenderness. The spleen was palpable 5 fingerbreadths below the left costal margin.

The hematocrit value was 16 per cent; icteric index 2; red blood cell count 2,100,000 per cu. mm.; hemoglobin 5.1 gm. per 100 ml. The white cell count was 4,400 per cu. mm. with 2 per cent myelocytes, 13 per cent juveniles, 55 per cent mature neutrophils, 5 per cent eosinophils, 1 per cent basophils, 17 per cent lymphocytes and 7 per cent monocytes. There was slight hypochromia of the red cells with anisocytosis and rare polychromatophilia. The urine was unremarkable. The serum uric acid was 16.4 mg. per 100 ml.; total serum proteins 9.6 gm. per 100 ml. with albumin 3.3 gm. per 100 ml. and globulin 6.3 gm. per 100 ml. Serum electrophoresis showed the albumin to be reduced and there was a heavy spot of abnormal protein in the β-2 globulin area. Fecal urobilinogen was 1960 Ehrlich units per 100 gm. of feces, an extremely high value. Direct Coomb's test was negative. Bone x-rays were unremarkable.

Bone marrow aspiration was done. The smears showed a large number (30 per cent) of mature and immature plasma cells and were considered diagnostic of multiple myeloma.

Despite transfusions of whole blood the hematocrit value did not rise above 26 per cent. It was felt that the patient had developed a significant hemolytic process and he was placed on 60 mg. of Prednisone per day. On this therapy there was considerable improvement and stabilization of the hematocrit value. The uric acid returned to a normal value of 5 mg. per 100 ml. The steroid dose was gradually reduced to 20 mg. per day and the patient was discharged.

Despite continuation of the Prednisone therapy, the hemolytic anemia persisted after discharge and could not be controlled with repeated transfusions and increased steroid dosage. The terminal episode was accompanied by development of furuncles on the buttocks, arms and forehead. Postmortem examination was not permitted.

M. E., JHH #724808 was a 49 year old white female housewife who was admitted December 14, 1955, with the chief complaint of weakness and shortness of breath.

Eighteen months prior to admission she noted weakness, easy fatigability, easy bruisability and shortness of breath. She had several episodes of spontaneous epistaxis. Because of increasing dizziness and shortness of breath she was admitted to her local hospital where she was found to have a low platelet count and anemia. It was very difficult to crossmatch her blood because of clumping of the red blood cells. She was begun on cortisone 25 mg. four times a day and referred to the Johns Hopkins Hospital.

Physical examination revealed a few small hemorrhages in the right fundus, questionable hepatomegaly and pallor but was otherwise normal. Laboratory data revealed a significant anemia with an hematocrit of 13 per cent. The white count was 7,850 per cu. mm. with 1 per cent differentiated myelocytes, 12 per cent juvenile neutrophils, 47 per cent segmented neutrophils, 2 per cent eosinophils, 27 per cent lymphocytes, 7 per cent monocytes, 4 per cent atypical lymphocytes and one nucleated red blood cell per 100 WBC. There was rouleaux-formation of the red blood cells. The platelet count was 112,000 per cu. mm. The serum non-protein nitrogen was 44 mg. per 100 ml.; total serum proteins 12.5 gm. per 100 ml. with 2.8 gm. per 100 ml. albumin and 9.7 gm. per 100 ml. globulin. Serum electrophoresis showed a broad band of abnormal protein occupying the gamma globulin area and the albumin was reduced. Serum calcium was 11.6 mg. per 100 ml. and the phosphorus was 3.2 mg. per 100 ml. There was no Bence Jones protein in the urine.

X-rays of the bones showed them to be slightly osteoporotic; however, it was noted

that the bones had a peculiar mottled appearance. The picture was produced by small islands of increased bone density located throughout the generally osteoporotic bone and had the appearance of actual osteosclerosis (Fig. 1). The report of the radiologist was as follows: "The peculiar increase in density of the bones suggests myelofibrosis usually seen with agnogenic myeloid metaplasia."

A bone marrow aspiration had been attempted on two occasions elsewhere but no marrow could be obtained. Thus, a bone marrow biopsy was performed which showed

FIG. 1. X-ray of the right iliac crest (area of biopsy in Case 2) showing osteoporosis and islands of sclerotic bone (skull x-rays normal at this time).

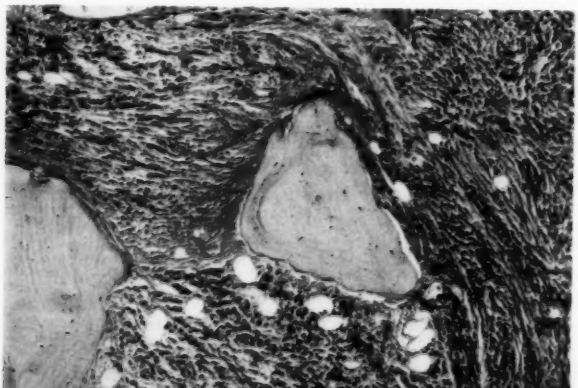

FIG. 2. Iliac crest bone marrow biopsy in Case 2 showing complete replacement of the marrow by fibrous tissue and plasma cells.

FIG. 3. Skull x-ray showing the typical osteolytic lesions of multiple myeloma (seven months after the x-ray in Fig. 1).

replacement of the normal marrow with fibrous tissue enmeshed in which were numerous plasma cells. These cells in some areas showed the typical eccentric nucleus and cartwheel appearance and in other areas they showed considerable pleomorphism and anaplasia. No normal marrow could be seen, the replacement by plasma cells and fibrous tissue being complete (Fig. 2). The diagnosis of multiple myeloma with myelofibrosis was made.

Therapeutic measures consisted of transfusions and continuing steroid medication. Subsequently, she was admitted to the hospital complaining of weakness, and despite continued steroid medication she required frequent transfusions. Seven months after her initial admission, the spleen was palpable for the first time.

Follow-up x-rays of the bones taken during the course of her disease showed the typical osteolytic lesions of multiple myeloma in the skull (Fig. 3). An x-ray of the right iliac crest ten months after the onset of symptoms showed further progression of osteoporosis but islands of sclerotic bone were still visible.

Thereafter her course was gradually downhill and her final admission was for treatment of uncontrollable gastrointestinal and vaginal bleeding. Terminally she was found to have positive blood cultures for para-colon bacilli. Postmortem examination was not permitted.

DISCUSSION

The coincidence of multiple myeloma with polycythemia has not received wide recognition in the medical literature. Lawrence and Rosenthal (4) reported the first cases that were adequately described and refer to the previous case reports of Pribram (5), Perla and Biller (6), Arnholt (7) and Tsuji (8) in which the diagnosis of both polycythemia vera and multiple myeloma was suggested but was never clearly established.

The four cases of Lawrence and Rosenthal included one case of secondary

polycythemia. In their cases the diagnosis of multiple myeloma was made either by postmortem examination or sternal marrow aspiration.

Carston and Ackerman (9) in 1955 reported the case of a 56 year old white male with polycythemia vera and a solitary bone defect seen on x-ray; he developed multiple osseous lesions and died 3½ years after the onset of symptoms. Unfortunately, the case history was not detailed enough in their review to be sure that this was a definitely proven case of multiple myeloma.

Lawrence and Donald (10) reviewed the literature on malignancies coexistent with chronic leukemia and polycythemia vera and mention one case of multiple myeloma with polycythemia vera but the case history is not given in detail.

A very well studied case is that of Giertsen (11) reported in 1956. His patient was a woman in her sixties who presented with a ruddy complexion and a cyanotic appearance of the extremities. There was no hepatosplenomegaly. The red blood cell count was 8.9 million. The white count was 9,100. She was treated with venesections and roentgen ray therapy to the sternum. Seven years later she was seen again with hepatosplenomegaly. Again she had the hematological findings of polycythemia vera. A sternal marrow examination revealed an increased number of plasma cells, some with loose cytoplasm and vacuoles. On another hospitalization she was found to have circumscribed bone defects in the skull. Paper electrophoresis of the urine showed a high gamma globulin excretion. The patient died with uremia. The autopsy revealed a large spleen, liver and heart. The bone marrow contained small nodules consisting of "soft, brittle, red-brown tissue." Histological sections showed the structure of the spleen to be distorted by a cellular infiltrate composed of cells of the myeloid series and plasma cells. The kidneys showed changes consistent with the diagnosis of "myeloma kidneys."

In our case the polycythemia vera was present three years before the discovery of multiple myeloma and hemolytic anemia. Despite steroid medication and transfusions the patient died with overwhelming infection.

Multiple myeloma with myelofibrosis has been reported previously. The case reports of Hodler (12), Sharnoff *et al.* (13), Krainin (14), Galgano (15), Bailey (16) and Kohler (17) review the histories of patients who had histologically proven multiple myeloma and x-ray demonstrated or histologically proven osteosclerosis. The new bone formation in the cases of Galgano and Bailey developed shortly after x-ray therapy to the myelomatous lesions and probably should not be included in the group.

The two cases of Hodler (13) are well documented examples of multiple myeloma and myelofibrosis with x-ray demonstrated sclerotic lesions. Anemia, thrombocytopenia, leucopenia and immature leucocytes in the periph-

eral smear were prominent hematological findings. Nucleated red cells were also abundant as were plasma cells in the peripheral smear. Postmortem examination revealed extensive myelomatous infiltrates in the bone marrow as well as marked fibrosis and sclerosis of the bone tissue.

In our case the anemia, thrombocytopenia, abnormal globulinemia, and rouleaux-formation of the red blood cells initially suggested multiple myeloma before the bone marrow biopsy. The unusual features were the bone films which showed the mottled density of the bones of the skeletal structures with actually small islands of sclerotic bone scattered throughout the ribs and pelvis. Bone marrow biopsy (iliac crest) revealed diffuse fibrosis and plasma cell infiltration of the marrow tissue. The presence of nucleated red blood cells and immature leucocytes on the peripheral smear and later development of splenomegaly were similar to those findings seen in myeloid metaplasia or myelofibrosis. Repeated transfusions and steroid therapy failed to prevent the ultimate outcome, the terminal episode being uncontrollable bleeding and septicemia.

These case reports further substantiate the clinical impression that the primitive stem cell of the bone marrow may develop into various final cell types, whether they be erythrocytes, leucocytes, thrombocytes, plasma cells, osteocytes or reticulum cells. Overproduction of two final cell types, the erythrocyte and plasma cell in the same patient resulting in polycythemia vera and multiple myeloma can be found in our first case and in the cases of Lawrence and Rosenthal and Giertsen. Moreover, the same reasoning can be applied to the case of myeloma and myelofibrosis seen in Case 2 in which plasma cells, osteoblasts and reticulum cells are produced in greater than normal amounts. Other combinations of two or more members of the myeloproliferative disorders are well known, e.g., polycythemia vera associated with chronic myelogenous and leukemia and myelofibrosis (2).

The concept that myeloid leukemia, myelofibrosis, polycythemia vera and megakaryocytic myelosis are similar and represent variable manifestations of a proliferative process of the primitive mesenchymal cell is now widely accepted. That multiple myeloma should be recognized in this same light is suggested by these case reports.

SUMMARY

Two case reports are presented, the first an instance of multiple myeloma and polycythemia vera; the second an example of multiple myeloma with myelofibrosis.

The reasons for including multiple myeloma in the spectrum of diseases known as the myeloproliferative disorders are discussed.

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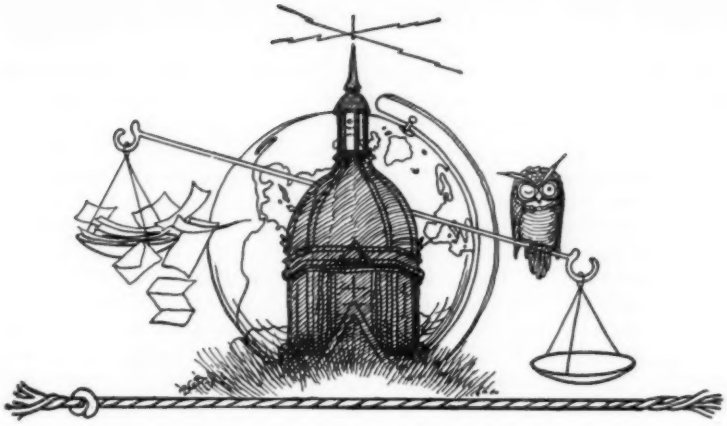

BOOK REVIEWS

Current Therapy 1960. Edited by HOWARD F. CONN. 808 pp., $12.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

This is the twelfth annual edition of this series of publications under the able editorship of Dr. Conn which has proven very helpful in the past to physicians in many branches of practice. Dr. Conn has made it the policy of this series to rotate each topic among several authors through the years, attempting to have each volume include both therapeutic measures long proven of real value as well as newer methods of established value or possessing significant promise. In some cases more than one author treats a given topic where there is room for difference of opinion. This policy makes available to the reader a wide scope of experience in each subject if volumes for several years are consulted and adds considerably to the overall value of the series, although each volume in itself has great merit through the competence of the consulting editors and individual contributors.

ROBERT E. MASON

Surgery in World War II. Neurosurgery Vol. II. Medical Department United States Army. Editors for Neurosurgery R. GLEN SPURLING and BARNES WOODHALL. 705 pp., $7.00. Superintendent of Documents, Government Printing Office, Washington, D. C., 1959.

This well illustrated volume of the History of Neurosurgery in World War II deals with 2 main topics, injuries of the spinal cord and the peripheral nerve injuries. There is also a chapter on lesions of the intervertebral disc included. Each chapter is introduced by a historical note written by Dr. Woodhall. The problems and the special policies are discussed in the zone of interior, in the Mediterranean theater of operations and finally in the European theater of operations in each of the two chapters. This gives a short but very interesting insight of the difficulties in organization and management of these patients.

There is an excellent chapter by Donald D. Matson on the management of acute compound battle-incurred injuries of the spinal cord and two equally informative and very practical chapters on urologic aspects of spinal cord injury by George C. Prather

and on the management of paraplegic patients in the zone of interior hospitals by Barnes Woodhall. Each of these authors has a large personal experience in his respective field and their expositions are colored by a convincing subjectivity. The approach to all these problems is very practical and didactic. Therefore, the book can be highly recommended to clinicians in the field of Neurology and Neurosurgery and to all practicing doctors. The management of the ruptured intervertebral disc by R. Glen Spurling is only a short account about the early and later policies in management in these cases during World War II.

Equally instructive and very informative is the chapter on peripheral nerve injuries. About two-thirds of the book is dedicated to this problem and after general discussion about early diagnosis, nerve grafting principles and techniques of the different procedures by Dr. Woodhall, Dr. Campbell, and Dr. Spurling, the consecutive presentations deal in a systematic way with the standard methods of examination in peripheral nerve injuries. The anatomic approaches to the more commonly injured peripheral nerves are presented by Warner Wells, Frederick M. Owens, Jr. and Francis A. Echlin. The different techniques and the nerve grafts are discussed in a lucid way by several authors and there is also a short survey of the causalgia following combat-incurred injuries of the peripheral nerves, by Dr. Frank H. Mayfield. The neuropathologic changes in the peripheral nerves are discussed by William R. Lyons. This chapter is very nicely documented with photo-micrographs mostly in black and white but some in color. Finally the physical therapy in the management of peripheral nerve lesions and also some of the orthopedic techniques for use in irreparable nerve injuries are discussed at the end of the book.

This book is an excellent effort to make available the accumulated personal experience of several outstanding specialists in the field of neurosurgery and applied disciplines and can be highly recommended to every one who is exposed to practical management of patients in these two difficult fields of neurosurgery.

GEORGE B. UDVARHELYI

Textbook of Otolaryngology. By DAVID D. DEWEESE and WILLIAM H. SAUNDERS. 464 pp., $8.75. The C. V. Mosby Co., St. Louis, Mo.

This is an extremely well written textbook, accompanied by excellent illustrations and diagrams covering all phases of ear, nose and throat. I think that the textbook is designed primarily for the medical student and general practitioner, rather than the trained otolaryngologist. Many of the discussions and presentations are more than adequate for learning the orientation for the medical student and are presented in such a way that it would be interesting rather than boresome.

The book begins with the complete discourse on the physical examination which is essential for the nose and throat diagnosis, and I think many points here are extremely simplified so that anyone who reads it could certainly get a great deal of information from it. The book then takes up the oral cavity and the pharynx and accompanying structures, including the larynx. There is a very interesting section on the trachea and the bronchi, trying to explain the instruments used for endoscopic work and also trying to give some understanding of pulmonary pathology and x-ray studies of the lung. The nose and sinuses are then discussed in a rather abbreviated form. The latter half of the book deals with the ear, and its associated problems, such as dizziness, vertigo, tinnitus, hearing loss, speech problems and so forth. The last chapter of the book deals with the salivary glands and associated infections and tumors, and again I think it is well presented.

I think that this book is well planned and organized for the purpose for which it was written. It is a most excellent book for learning, and as mentioned previously, for the medical student and the general practitioner, both of whom should have this textbook available. This will answer many problems which the general practitioner comes into contact with in his every day work and is so presented that it can be used by him with great ease. I like the fact that there is a great deal of emphasis on diagnosis and treatment and a good explanation of some of the new concepts of otolaryngology. Many of these are relatively new in their development, and I am very glad to see them incorporated within this book. Many of the recent developments in otologic surgery have been included with some aspects of the physiology of hearing correlated to the respective operative procedures.

I would like to compliment the authors on this fine textbook, and I am sure there will be a great need for it in the teaching centers throughout the country.

ALVIN P. WENGER

The Acute Medical Syndromes and Emergencies. By ALBERT S. HYMAN with the collaboration of SAMUEL WEISS, GEORGE G. ORNSTEIN, HOWARD F. ROOT, ANNA RUTH SPIEGELMAN and JACK ABRY. 422 pp., $8.75. Landsberger Medical Books, New York, N. Y.

This volume is designed, according to the editor, to afford the busy physician an opportunity to acquaint himself with recent advances in the diagnosis and treatment of the acute medical emergencies and to re-evaluate traditional concepts in the light of scientific progress in the various disciplines. The subject matter has been "allocated in accordance with the results of a nation-wide poll in which physicians in active practice were asked to list the type of important acute medical problems which they had been called upon to treat in the previous 5 years". This has resulted in 6 sections—each written by an expert in the field—dealing respectively with cardiovascular, gastrointestinal and pulmonary problems (the three most frequently encountered types of emergencies), and with acute diabetic problems, acute renal crises and barbiturate intoxication.

Following a discussion of the diagnosis of a specific acute problem, the suggested treatment is outlined with frequent reference to the theoretical rationale and to available statistical data relative to the results being obtained.

Certain features, including in places a rather general discussion in lieu of specificities, the frequent omission of dosages, the use of either trade or generic drug names (but frequently not both together) represent deficiencies which render this volume less useful to the harrassed intern in the Emergency Room or the practitioner for rapid reference. An exception is the hour-by-hour, perhaps necessarily dogmatic outline of the treatment of diabetic acidosis and coma which is organized under seven headings (the second of which is subdivided into 19 specific steps to be followed).

There are other deficiencies or controversial points such as the omission of any mention of the use of antihypertensive agents in the treatment of hypertensive encephalopathy and the implication that prothrombin function can be rapidly restored to normal (following anticoagulation) by any of the natural or synthetic vitamin K preparations. External cardiac resuscitation is too new to be included.

In general, however, this book should serve as a useful review to any physician who is likely to see patients with acute medical emergencies.

JOSEPH E. JOHNSON III

Pain and Itch, Nervous Mechanisms. Ciba Foundation Study Group No. 1. Edited by G. E. W. Wolstenholme and Maeve O'Connor, 120 pp., $2.50. Little, Brown & Co., Boston, Mass., 1959.

It is a real pleasure to review this little book which presents ideas and new approaches to one of the still unsolved problems of medicine and physiological thinking: pain and the allied sensation, itch. Its great value lies in the informal and very honest way in which this rather complicated topic is discussed.

The volume is dedicated to Professor Zotterman from Stockholm, Sweden and the contributors include several outstanding workers in the field of Neurophysiology. Chairman of this symposium is Lord Adrian who wrote the opening remarks and who also closes, with the modesty of real wisdom, the general discussion at the end of the book.

As can be expected most of the papers deal with rather specific problems of the technique of recording of different potentials and will be of considerable interest to the workers in this specific field. Professor Zotterman gives a very lucid brief review of the peripheral nervous mechanisms of pain. Iggo from Edinburgh gives a concise report on analysis of activity in single C fibers. His results apparently are sufficient to refute any hypothesis which asserts that all nerve endings with afferent C fibers are identical in their responses to cutaneous stimulation. He makes the important conclusion that the C fibers may have hitherto unrecognized importance both for reflex activity and for sensation. Gordon from Oxford discusses some patterns of activity in the central sensory pathway with possible relevance to the problem of pain. He makes it clear that there are differences in the main central conduction of velocities and also stresses the sparseness of hair units in the ipsilateral thalamus. There is a very stimulating discussion after this chapter. Equally interesting are the chapters by Weddell, Palmer and Taylor; Douglas and Ritchie; and Landgren.

The peripheral mechanism of itch in man is discussed by Arthur and Shelley; Kugelberg and Lindblom discuss the mechanism of pain in trigeminal neuralgia. These two last papers have interest also to clinicians.

The presentation of the papers together with the actual discussions make this little booklet very interesting reading, not only for researchers in this specific field, but also for students and clinicians.

George B. Udvarhelyi

BOOKS RECEIVED FOR REVIEW

Clinical Orthopaedics, No. 16. The Foot. Edited by Anthony F. DePalma. 315 pp., $7.00 single number. J. B. Lippincott Co., Philadelphia, Pa., 1960.

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SODIUM AND CHLORIDE AND ACID-BASE PHYSIOLOGY

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The last ten or fifteen years have witnessed a pronounced shift of descriptive emphasis in electrolyte physiology. Whereas, in the past, acid-base physiology has been presented almost exclusively in terms of alterations in the metabolism of the "fixed ions",[2] it is described now with primary emphasis upon renal excretion of hydrogen or reabsorption of bicarbonate. It is the purpose here to discuss certain problems engendered by this change and to clarify, in particular, the role of the fixed ions in the newer formulations.

The increased emphasis upon hydrogen ion excretion as the important determinant in acid-base physiology can be attributed, first, to change in terminology, and, second, to a generally accepted concept pertaining to the mechanism of urine acidification.

CHANGE IN TERMINOLOGY

The problem of terminology in "acid-base" metabolism is considered with the aid of the classification shown in Table I. This evaluation has been oriented rather specifically to deal with problems that have arisen with respect to the meaning of the word acid in clinical physiology. For more general and authoritative treatises on this topic the reader is referred to works of Dr. Mansfield Clark (1, 2).

The first entry lists HCl as an acid and NaOH as a base. This classification is that presented to students first in courses in elementary chemistry; and it becomes an indelible point of reference during ensuing years. In this sense, the acid and the base are separate substances with opposite properties.

In the second entry, chloride is referred to as "fixed acid" and sodium as "fixed base". These are older designations which found usefulness in clinical physiology on an *empirical* basis. For example, if the kidney engineers an increase in the excretion of chloride relative to that of sodium, an increased excretion of acid obtains which can be related quantitatively to the appearance of the excess chloride, in the urine. Changes in the concentration of chloride in biological fluids correlate positively

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[2] "Fixed" is an older term meaning not evolved or destroyed. It is used here to designate all of the inorganic ions except H^+, OH^-, and NH_4^+.

TABLE I
Terminology

	Acid	Base
Elementary Chemistry	HCl	NaOH
Older Usage (Clinical Physiology)	Cl^- is a "fixed acid"	Na^+ is a "fixed base"
Lowry-Brønsted	Hydrogen Ion Donor Proton-Proton Acceptor (e.g. HAC, acetic acid)	Hydrogen Ion Acceptor Proton Acceptor (e.g. AC^-, acetate ion)
A Modern Usage (Clinical Physiology)	"Hydrogen, H, or Hydrogen ion excretion" to designate acid excretion	

with acidity. Conversely, changes in the content of sodium correlate positively with alkalinity. Recently, however, the usage of these terms has come under a good deal of editorial fire (3, 4, 5, 6) because of the marked discrepancy between this concept and that accepted by the majority of today's chemists.

At this time, the most widely recognized definition is that set forth by Lowry and by Brønsted in 1923 (7, 8). As stated by Brønsted, "An acid is a substance that can lose a hydrogen ion, thus forming a base, while conversely a base is a substance which can gain a proton, thus forming an acid."[3] It might be noted parenthetically that here the base is the "stem" of the acid, actually the whole of the molecule except the ionizable hydrogen. In comparison, the base of the first listing is a separate substance that neutralizes acid but never becomes part of the acid molecule.

Modern authors have recognized the need to employ generally understood terms, and a sincere effort has been made to adopt the concepts of Lowry and Brønsted to the problems of clinical physiology. It is recognized that acidification of the urine should be described by referring to additions of hydrogen ions (protons) to urine buffers (proton acceptors). However, the changeover to this view has not been achieved without encountering certain pitfalls. Concern is expressed over the extremely loose usage of the word hydrogen which has resulted in failure to distinguish, in generally recognized terms, between monumental quantitative differences. For example, the phrases "excretion of hydrogen" or "loss of hydrogen from the cells" are not definitive for description of the transfer of acid. It is obvious that atoms of hydrogens in molecules of water and organic substances are being transferred continuously in thousand-fold larger quantities than can be associated with the formation or movement of acid.

[3] The eventual hydrogen donor of an aqueous solution of hydrochloric acid is considered to be the hydronium ion, $H_2O \cdot H^+$ ($HCl + H_2O \rightarrow H_2O \cdot H^+ + Cl^-$).

Care should be exercised when using the expression "hydrogen bound to buffers" to specify only the increments bound; for many buffers such as citric acid have hydrogen attached in forms that do not participate in the buffering reaction. The phrase "excretion of hydrogen ions" can also be confusing. Acid excretion can be related quantitatively to the number of protons added to buffers at the tubule membrane; but, once added, these protons become tightly bound. In a given volume of urine the trace amount of ionized hydrogen is obviously insignificant in relation to the total quantity of excreted acid. The expressions referred to in quotations above are employed frequently, and the intended significance with reference to quantity is understood in the special field. As with "fixed acid" and "fixed base", however, the designations are puzzling to workers of other disciplines.

It can be seen that reorientation away from a viewpoint based primarily upon the metabolism of the fixed ions has posed new problems with respect to quantitative description. Nevertheless, if the Lowry-Brønsted concept is to be adopted, events in acid-base physiology must be described in terms of alterations in the concentrations or activities of the various proton donors and proton acceptors. Since the strong electrolytes, such as sodium or chloride, neither donate nor accept significant quantities of protons in the physiological pH range, they do not qualify in this view as either acid or base.

MECHANISM OF URINE ACIDIFICATION

A second cause for emphasis upon hydrogen ion excretion in today's literature can be attributed to widespread utilization of postulations that were set down originally to explain the mechanism of action of the drugs such as acetazolamide (Diamox). Administration of potent inhibitors of the carbonic anhydrase reaction induce an increased excretion of sodium and a corresponding decrease in urine acidification. It is believed that the rate of the carbonic anhydrase reaction is critical in regard to supply of hydrogen ions for transport into the lumen to form urine acid; and, furthermore, that this limitation upon the availability of intracellular protons for excretion will restrict, as a secondary effect, the reciprocal movement and hence reabsorption of sodium. This theory as proposed by Pitts (9) is an extremely important one. It should not, however, monopolize our thinking in regard to identification of the primary events at the membrane level. Acidification is an active process requiring energy input and active transport. Theoretically, "hydrogen for sodium exchange" can be motivated either by active secretion of hydrogen ions or by active reabsorption of sodium ions. It is emphasized here that an acetazolamide-

induced change in intracellular pH would be expected to have a direct effect upon sodium transport. This is expected because of the known importance of pH upon enzymatic activity and cellular metabolism generally. In addition, reference is made to observations by Skou and Zerahn of the importance of pH in active transport of sodium cross the isolated frog skin (10). These authors associated marked changes in the rates of sodium transport with pH determined alterations in the degree of ionization of certain organic amines in the incubation medium.

In short, the possibility of a direct effect of pH change upon the mechanism of active reabsorption of sodium is underlined and is considered here as a supplementary reaction involved in the renal response to acetazolamide. As stated previously, if definition is the prime requisite, aspects of hydrogen ion transfers should be acknowledged in descriptions of acid-base physiology. On the other hand, if the primary aim is the elucidation of mechanisms of acid excretion, one must recognize that hydrogen ion excretion and bicarbonate ion reabsorption are part and parcel of complex reactions which may be motivated by active transport of fixed ions.

PRACTICAL ASPECTS AND CLINICAL SYNDROMES

There exists today a rather curious double approach to analyses of electrolyte problems. When a clinician is evaluating a particular patient at the bedside, he finds himself referring to the disorder in terms of deficits and surpluses of the fixed ions; and yet when he sits down at night to read the literature of clinical investigation, he comes upon many descriptions presented in terms only of disorders in the excretion of hydrogen ions or reabsorption of bicarbonate ions. In actuality, as has been emphasized (11), these two approaches are merely the opposite sides of the same coin. Reciprocal relationships in the excretion-reabsorption patterns of fixed and of other ions must obtain to balance the overall equations in compliance with the law of electroneutrality. It has been convenient in the past to describe clinical syndromes in terms of balances between the intake and the output of the strong electrolytes. These exist predominantly in ionized form and retain measurable identity in all of the body fluids. The hydrogen nuclei, in contrast, do not exist in the dissociated form in tangible concentrations, but are bound tightly by buffers; and these vary in type in the different biological compartments. Balances of protons are necessarily more difficult to measure and to describe, and they have been assembled with a corresponding infrequency.

At this point it is worthwhile to consider the two sides of the coin with reference to certain clinical problems. It is useful, for the present purposes, to discuss the etiology of hyperchloremic acidosis of renal origin and of

hypochloremic alkalosis due to vomiting. In practice the abnormality in these conditions is described by placing emphasis upon the metabolism either of hydrogen ions or of chloride ions. The term hyperchloremic acidosis places emphasis upon an easily recognized entity, the high serum chloride; and, indeed, an abnormal increase in chloride reabsorption may be the primary disorder. Since, however, chloride is not an acid, further comment is required to explain the disturbance in acidity. It must be pointed out that an increased reabsorption of chloride will specify, in the balanced equation of the overall reaction, a decrease in the reabsorption of bicarbonate. A decrease in the extracellular concentration of this and of other proton acceptors will effect a lowering of the extracellular pH in accordance with relationships as exemplified by the Henderson-Hasselbalch equation. At the present time, however, it is more usual to turn this side of the coin downwards and to place the primary emphasis upon a decreased transfer of hydrogen ions across the tubule cell membrane to urine buffers. Retention of these hydrogen ions will be reflected by a decrease in the body fluids of the ratios of proton acceptors to proton donors. This amounts to a using up, so to speak, of the available buffering capacity or of "buffer base" (11, 12). This descriptive approach can be recommended because of the direct utilization of the principles of Lowry and Brønsted, but it tends to minimize the possible etiological importance of abnormalities in fixed ion metabolism.

The second syndrome, hypochloremic alkalosis of vomiting, provides a somewhat more obvious example of the need to maintain the correct amount of chloride in the extracellular fluids. The physical chemistry of the alkalosis can be ascribed to a decreased availability of proton donors for reaction with a proton acceptor excess. The deficiency of chloride, however, is a very real one. No amount of hydrogen ion given as organic acid can restore health. This problem is considered in more detail with reference to a special experiment.

CHLORIDE AS A FUNDAMENTAL UNIT OF ELECTROLYTE STRUCTURE

In this study (Fig. 1) a dog was first "primed" by a diet high in chloride. He was then offered, for a period of six days, only 20 mEq. each of sodium and potassium bicarbonate dissolved in a liter of 10 per cent glucose. On the first day, following the abrupt removal of chloride from the diet, the animal excreted 37 mEq. of this ion. As shown in the chart, the loss was associated with a rise in the plasma pH and CO_2 content which was sustained through the 6th day. At this point the elevations can be attributed either to bicarbonate loading or to chloride deficiency. After six days, the intake was supplemented with 2.34 grams of sodium chloride (40 mEq.), and recovery ensued promptly. This recovery took place without change

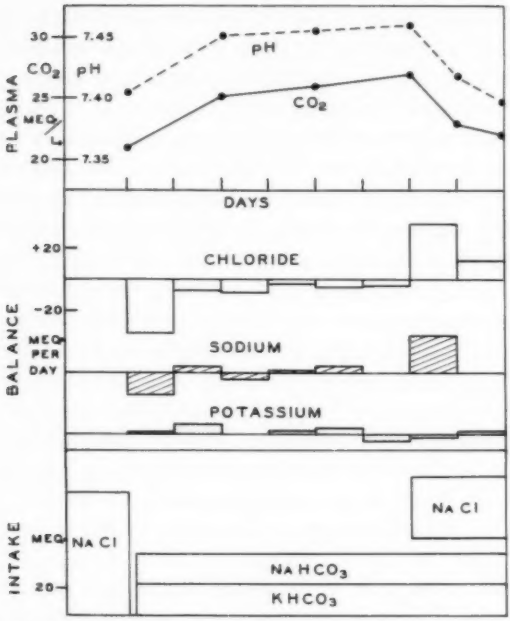

FIG. 1. Effect of chloride depletion and replenishment upon the serum CO_2 content and pH and upon the balances of sodium, potassium, and chloride. The procedures and methods used have been described in a previous communication (18). The dog's food and water allowance was limited to 1000 ml. of 10 per cent glucose per day.

Corrections have been applied to the measured potassium balances to account for losses excreted in association with a negative nitrogen balance.

in the intake of bicarbonate or of "hydrogen", and it was associated with the indicated positive balance for chloride. These events point to the deficiency of the fixed ion as the critically important factor.

Clinical physiologists have recognized that saline is therapeutic in mild to moderate, (13, 14, 15, 16) but not in severe (16, 17) instances of metabolic alkalosis in which hormonal effects and potassium deficiency become important factors (18, 19).

PHYSIOLOGY AND PHYSICAL CHEMISTRY

It is frequently not enough for the physiologist to designate acid excretion in terms only of increased addition of hydrogen ions to urine buffers. Excretion of a given equivalence of acid might indicate, in one patient, an abnormal loss from the extracellular fluids with increasing alkalosis. In a second patient, the same loss could be due simply to a normal overflow of acid products as a direct reflection of the intake. Implications with

respect to mechanisms in these two instances are somewhat different. Abnormal loss of acid can be due, theoretically, to fundamental defects in tubule cell metabolism resulting in hyperexcretion primarily of hydrogen ions or primarily of chloride ions. On the other hand, when anions such as phosphate or sulphate are lost in amounts that exactly balance the intake, it would appear that it is these anions specifically that are the targets of the control mechanisms. Here the reciprocal movements of hydrogen ions during excretion, or for that matter during absorption, would appear to be secondary.

IN CONCLUSION

Optimally, descriptive analyses of changes in acid-base metabolism should relate directly to the primary motivating mechanisms, but unfortunately these cannot be identified at this time. It is not known whether the balance is maintained primarily by hydrogen ion secretion and bicarbonate ion reabsorption; or whether, on the other hand, the control is exerted by regulated secretion and reabsorption of certain fixed ions. At this stage it would appear an oversimplification for the physiologist to direct all attention towards only one or two of the components. Lawrence Henderson (20) once wrote "Life itself can be considered as a physical-chemical system in which there occur many variables in a state of mutual dependence". It seems wisest, with respect to regulation of the pH of the extracellular fluids, to continue to recognize interdependence between many components, realizing that an aberration in the control over anyone may be of the utmost importance in a given syndrome. If the fixed anions and cations are considered, not as acids and bases respectively, but as essential reactants in the overall equations that control the excretion of acid, no incompatibility with modern concepts will develop.

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SHUNTING EFFECT OF EXTREME IMPAIRMENT OF PULMONARY DIFFUSION[1, 2]

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Impaired pulmonary diffusion is observed in a number of clinical conditions, usually grouped together under the "alveolar-capillary block syndrome." The pathologic physiology of this entity has recently been studied by several investigators (1 to 3). The usual findings include a normal or low arterial carbon dioxide tension, a normal or elevated alveolar oxygen tension, and a somewhat reduced arterial oxygen tension with a normal or near normal arterial oxyhemoglobin saturation in the resting condition and breathing air.

Although impairment of O_2 diffusion is the chief physiologic abnormality, there is usually also a certain degree of uneveness in the ventilation-perfusion ratios (2, 4) and in the moderately severe cases, the magnitude of the two abnormalities can be separately estimated (2, 5).

This paper deals with the special problems in analyzing the physiologic disturbances in the cases where impairment of diffusion has reached extreme degrees of severity.

SUBJECTS AND METHODS

Five patients with advanced pulmonary disease limited to the alveolar-capillary bed were studied. The diagnoses, obtained at autopsy or by lung biopsy were: pulmonary alveolar proteinosis (Case 1), cryptococcus pneumonitis (Case 2), severe interstitial fibrosis (Cases 3 and 4), and pulmonary pneumocystosis (Case 5).

Physiologic studies were performed with the patients resting in the semi-recumbent position. Arterial blood was drawn from the brachial artery using an indwelling Riley needle. Blood was analyzed for O_2 and CO_2 tensions by the modified bubble equilibration method of Riley, *et al.*, (6) and for O_2 content, O_2 capacity and whole blood CO_2 content by the manometric technique of Van Slyke and Neill (7). Arterial blood pH was measured in a closed Cambridge glass electrode at 37°C. In two patients, expired gas was simultaneously collected in a Tissot respirometer and was analyzed for O_2 and CO_2 in the Scholander 0.5 ml. gas analyzer. Measurements were also made on four subjects while breathing high O_2 mixtures and in one case with the subject breathing a low O_2 mixture (16.26 per cent

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TABLE I

Arterial Blood O_2 Saturation in Five Patients with Severe Impairment of Diffusion

Case No.	Saturation, on Air	Saturation, breathing O_2
	%	%
1	66	91 (mask)
2	49	90 (mouthpiece)
3	74	
4	85	
5	58	

O_2 in N_2). The study at two levels of oxygenation (Case 4) permitted fractionation of the alveolar-arterial P_{O_2} gradient into its diffusion and venous admixture components, and calculation of the pulmonary diffusing capacity (5). In the other cases, an estimate of the degree of venous admixture was made according to the method discussed in the Appendix. Since mixed venous oxygen saturation was not known, a difference of 20 to 30 per cent oxygen saturation was assumed between arterial and mixed venous blood. It has been shown that such an assumption is unlikely to introduce significant errors in the calculation of the venous admixture ratios or of the diffusing capacity (2). The "effective" alveolar oxygen tension ($P^e_{A_{O_2}}$) was calculated from the inspired oxygen tension ($P_{I_{O_2}}$), alveolar CO_2 tension[3] ($P_{A_{CO_2}}$), and the CO_2–O_2 exchange ratio (R):

$$P_{A^e O_2} = P_{I_{O_2}} - \frac{P_{A_{CO_2}}}{R} + \frac{P_{A_{CO_2}}}{R} F_{I_{O_2}} (1 - R)$$

Physiological dead space was calculated from the arterial P_{CO_2} (corrected for venous admixture), the expired P_{CO_2} and the tidal volume according to the Bohr equation. Correction was made for the apparatus dead space as measured by water displacement.

RESULTS

A summary of gas exchange findings in the five subjects is shown in Tables I and II.

I. Arterial O_2 Saturation

In the resting state and breathing ambient air, there was a remarkably low arterial oxygen tension and oxyhemoglobin saturation in all cases.

Breathing pure O_2 in Cases 1 and 2 resulted in some increase in arterial O_2 saturation but failed to correct the hypoxia completely. Pure O_2 breathing brought the saturation up to 98 per cent in Case 4. Arterial O_2 saturation was very low in Case 5, breathing 28 per cent O_2. No physiologic measurements were made on Case 3 while breathing oxygen.

[3] Since in many of the determinations in this study there was a greatly increased venous admixture, a significant (>2 mm. Hg) arterial-alveolar P_{CO_2} gradient probably existed. Correction of arterial P_{CO_2} for venous admixture was therefore necessary for the calculation of venous admixture, diffusing capacity and dead space (5).

TABLE II

*Distribution and Diffusion Data in Five Cases with Severe Impairment of Diffusion**

Case No.	Date	$\dot{Q}_{va}/\dot{Q}_t \times 100$†	$V_D/V_T \times 100$	D_{LO_2}
				ml. per min. per mm. Hg
1	12/9/58	50–60		
	12/15/58	46–56		
	12/17/58	29–39		
	1/5/59	21–29	34	
2	10/16/58	47–57		
	10/21/58	63–71		
3	12/12/59	43–53		
4	6/10/59	28	62	6
5	10/26/59	58–68		

* Symbols:

$\dot{Q}_{va}/\dot{Q}_t \times 100$ = venous admixture ratio, per cent of cardiac output.

$V_D/V_T \times 100$ = physiologic dead space per tidal volume ratio, per cent.

D_{LO_2} = apparent pulmonary diffusing capacity for O_2.

† Estimated by the method of Riley (5) in Case 4. In all other cases venous admixture was calculated as described in the Appendix.

II. Diffusion and Distribution Data

Pulmonary diffusing capacity for oxygen was measured in Case 4 and found to be very low. Estimates of diffusion were not made in the other cases. However, a comparison of the clinical, physiologic and pathologic findings permits the assumption that diffusion was seriously impaired in all cases. Case 4, with an O_2 diffusing capacity of 6 ml. per min. per mm. Hg, appeared to be of somewhat less severity than that of Case 1, Case 2 or Case 5, and was perhaps comparable in severity to Case 3.

Venous admixture,[4] expressed as a percentage ratio of the cardiac output ($\dot{Q}va/\dot{Q}t \times 100$) was markedly increased in all cases. In Cases 1 and 2, where serial estimations were made during the course of the disease, the degree of venous admixture was found to correlate well with the clinical picture. $\dot{Q}va/\dot{Q}t \times 100$ reached the high values of 50–60 and 63–71, respectively, at the peak of symptoms, and gradually declined toward normal with clinical improvement.

Physiological dead space was measured in Cases 1 and 4 and found to be elevated ($V_D/V_T \times 100 > 30$). In Case 1, a moderately increased dead space was found even after appreciable symptomatic improvement.

[4] See Appendix.

DISCUSSION

Effect of Impaired Pulmonary Diffusion on Arterial Oxygen Saturation

The manner in which diffusion can affect the O_2 saturation of the pulmonary capillary and of arterial blood has been studied and analyzed by Bohr (8), and more recently by Riley and Cournand (9) and others (3).

Normally, and in the resting state, breathing air is sufficient to bring the O_2 pressure in the "end-capillary" blood to almost complete equilibrium with that of the alveolar gas. Even with marked lowering of the pulmonary diffusing capacity, the "end-capillary" blood O_2 saturation decreases but little although varying degrees of exercise will cause a drop in the saturation of the "end-capillary" and arterial blood. The drop is usually a precipitous one and tends to occur at decreasing levels of O_2 consumption with increasing deterioration of diffusion (10). In the average case of diffusion difficulty, this drop in saturation can be corrected by breathing O_2 in concentrations only moderately higher than that of air.

Shunting Effect of Severe Diffusion Impairment: The unusually low arterial oxygen saturation in our subjects and the failure of pure oxygen breathing to correct the hypoxia completely could not, therefore, be explained on the basis of a generally reduced diffusing capacity *per se*. The results could, however, be explained by postulating a mechanism whereby a large volume of venous or poorly oxygenated blood was shunted across the lung. The question now is whether a process which primarily affects diffusion could result in such a mechanism. This is believed to be possible if portions of the alveolar-capillary bed were affected by disease in such a manner as to render them virtually impermeable to any form of gas exchange while remaining relatively well-perfused with blood. The situation would then resemble one where venous blood from these segments of the alveolar-capillary bed was shunted across to the arterial side without actually coming in contact with alveolar gas. The pathologic lesions in the five cases under discussion were of a nature and an extent that could lead to such a situation. In Cases 1, 2 and 5, the alveolar spaces were filled with proteinaceous material or inflammatory exudate. It is likely that O_2 diffusion through these alveoli was practically impossible. Similarly, in Cases 3 and 4, there was extensive interstitial fibrosis and many alveoli were lined by several layers of thick epithelium.

In dealing with cases where the diffusion difficulty is severe enough to offer a nearly complete barrier to gas exchange in some areas of the diffusing surface, it may be useful to consider the situation as equivalent to one where shunting of blood occurred across portions of the alveolar-capillary bed, the severity of the case being determined by the relative size of these shunting portions.

It is obvious, therefore, that in extreme impairment of diffusion it becomes pointless to consider as separate the effects of poor diffusion and those of poor distribution since, in actual fact, the poor distribution (large venous admixture) is the inevitable result of a severely impaired diffusion. Riley points out that "one is forced to the conclusion that the very concept of impaired diffusion is indistinguishable from the concept of impaired distribution in extreme cases" (11). For this reason, calculations based on the accurate fractionation of the A-a P_{O_2} gradient into a diffusion and a venous admixture gradient (such as the equation for the estimation of venous admixture) become untenable in their usual form. A suggested modification of the formula for calculating "venous admixture" under these conditions is included in the Appendix.

SUMMARY

1. The physiologic effects of extreme impairment of pulmonary diffusion have been analyzed in five cases of "alveolar-capillary block."

2. The physiologic effects of extreme impairment of pulmonary diffusion are predominantly those of a large right-to-left shunt.

3. A revised approach is suggested for the calculation of venous admixture when the latter results from extreme diffusion impairment. The approach is based on taking the entire A-a gradient and calling it venous admixture.

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APPENDIX

Calculation of the Shunt Effect in Extreme Diffusion Impairment

The degree of venous admixture, expressed as a per cent ratio of the total pulmonary blood flow ($\dot{Q}va/\dot{Q}t \times 100$), is a useful estimate of the measure to which blood leaving the pulmonary capillaries is mixed with venous or poorly oxygenated blood before it reaches the left side of the heart. The source of venous or poorly oxygenated blood may be an actual "anatomical" shunt or a virtual "apparent" shunt resulting from uneven alveolar ventilation in relation to perfusion. The formula for calculating the shunt is:

$$\frac{S^{e}_{cO_2} - S_{aO_2}}{S^{e}_{cO_2} - S_{\bar{v}O_2}} \times 100 = \frac{\dot{Q}va}{\dot{Q}_t} \times 100 \qquad (1)$$

where $S^{c}_{\bar{c}O_2}$ is the O_2 saturation in the pulmonary end capillaries, S_{aO_2} is the arterial O_2 saturation and $S_{\bar{v}O_2}$ is the mixed venous O_2 saturation. The numerator of equation (1), expressed in terms of partial pressure, i.e., $P^{c}_{\bar{c}O_2}-P_{aO_2}$ is the venous admixture or distribution gradient. The remainder of the alveolar-arterial O_2 gradient, i.e., $P^{c}_{AO_2}-P^{c}_{\bar{c}O_2}$ represents the diffusion component of that gradient. Normally, or even in the presence of fairly severe limitation of diffusion, the diffusion gradient forms but a small fraction of the A-a gradient in the resting state and breathing air.

Accurate fractionation of the A-a gradient can be made by performing studies at two levels of oxygenation which accentuate the effects of one component or the other (5). When diffusion limitation reaches extreme degrees of severity, however, the effects of poor diffusion merge with those of poor distribution to such an extent as to make their separation difficult. Furthermore, since in these cases the impairment in diffusion and in distribution result from the same pathologic process, a study of the summation of the two effects would be more meaningful in evaluating the severity of the disease process. The combined effect would be reflected in the drop in P_{O_2} from the effective alveolar point to the arterial point. In other words, the entire A-a gradient could be taken to represent "venous admixture" in these cases.

For a mathematical expression of this expanded concept of venous admixture, equation (1) could be modified to read:

$$\frac{S^{A}_{\bar{c}O_2} - S_{aO_2}}{S^{A}_{\bar{c}O_2} - S_{\bar{v}O_2}} \times 100 = \frac{\dot{Q}va}{\dot{Q}_t} \times 100 \qquad (2)$$

where $S^{A}_{\bar{c}O_2}$ is the saturation that the blood would have if it were in complete equilibrium with the alveolar oxygen. It is obtained from the alveolar oxygen tension by reference to the O_2 dissociation curve.

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SOME OBSERVATIONS ON THE STAPHYLOCOCCUS IN A PEDIATRIC OUT-PATIENT POPULATION[1]

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INTRODUCTION

In recent years epidemics of staphylococcal disease have stimulated extensive investigation of the distribution of the staphylococcus in specific population groups. Results of these studies indicate that human colonization with staphylococci is more common in hospital communities such as maternity and nursery units than in non-hospital groups (1). It has also been demonstrated that the rate of staphylococcal nasal carriers increases in student nurses as the degree of hospital contact becomes greater (2), and in patients as the duration of hospitalization is prolonged (3). Moreover, the frequency of occurrence of penicillin resistant staphylococci increases with the duration of hospitalization (4).

The increasing incidence of penicillin resistant organisms in hospital populations has led to the substitution of newer antistaphylococcal chemotherapeutic agents in situations where penicillin had previously been used. Furthermore, fear of penicillin resistant strains in non-hospital populations has led to the greater use of these newer agents by staffs of out-patient clinics and by physicians in practice.

A study was undertaken, therefore, to evaluate the prevalence of staphylococci in the nares of children seen in the general pediatric out-patient department of the Johns Hopkins Hospital. Previous studies of out-patient populations (5, 6, 7) have concerned adults or selected groups of children (those admitted for elective surgical procedures). In addition, this study was designed to evaluate what effect hospital contact and/or treatment with penicillin had upon the likelihood of an individual's harboring antibiotic resistant staphylococci.

MATERIALS AND METHODS

Two hundred and five children varying in age from one week to 14 years who were seen in the general out-patient clinic of the Harriet Lane Home were studied during

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January and February, 1959. The children presented with a variety of complaints, and accordingly consisted of individuals with obvious infection and individuals in whom no infection was detected. In an attempt to select children representative of those seen in the general out-patient population, ten children arriving consecutively during a three-hour period three days a week were studied.

Interviews: To determine the extent of hospital contact and penicillin exposure each child's medical record was examined and the accompanying parent or adult was questioned in regards to the following:

1. Frequency of visits to the out-patient departments.
2. Time of last antibiotic treatment, with particular reference to penicillin.
3. Presence of hospital employees or frequent clinic visitors in the child's household.
4. Place of birth of the patient and his siblings if under six months of age.
5. If the patient was born in another hospital, length of stay in that hospital.
6. Time and duration of previous hospital admissions.

To define the degree of hospital contact a variety of factors were assessed in all individuals. Each patient was graded from 0 to 4, corresponding to an increasing degree of hospital contact, on the basis of the following definitions.

1+ . . . two out-patient department visits in one month.

1–3+ . . . more than two out-patient department visits in one month, depending on frequency.

0–2+ . . . if hospital employee in the household, depending on position in regard to exposure to patients.

3–4+ . . . in-patient admissions and hospital birth if within six months of this study, depending upon length of stay and proximity to time at which culture was taken.

If several areas of hospital contact were applicable to one individual the scores were added to one another and the total was used to represent the degree of hospital contact. Arbitrarily, individuals with totals of three or more were defined as having "significant hospital contact", while those with less than a three total were considered not to have had significant contact with the hospital.

Similarly, only those individuals who had received penicillin within the preceding six months were considered to have had a significant exposure to penicillin. There were five infants who were born in other hospitals after a complicated delivery (premature rupture of membranes, prematurity) who had prolonged stays in the nurseries. Because of the common usage of antibiotics in such children these infants were assumed to have received penicillin.

Cultures: Specimens for culture were obtained from each patient according to the following routine.

(1) In each subject over two years of age, a culture was obtained from the anterior nares (8).

(2) In each subject under two years of age, a wire cotton tipped swab was inserted into the nasopharynx and withdrawn in such a way that the anterior nares were also swabbed. This variation was used because of the difficulty in obtaining adequate anterior nares cultures on young children.

(3) In each patient with a purulent lesion, a culture of the pus was obtained.

Specimens were promptly cultured on blood agar and in trypticase soy broth and incubated at 37° for 36 hours. Any staphylococci present, irrespective of pigment production or prevalence, were isolated in pure culture. If two different types of staphylococcal colonies were identified each was isolated separately.

Following isolation, antibiotic sensitivity of the staphylococcus was determined using

paper discs. All staphylococci isolated were examined for coagulase activity using the method of Schaub (9). The coagulase positive strains were typed with bacteriophage according to the method of Blair (10) with modifications as recommended by the Subcommittee of Bacteriophage Typing of Staphylococci of the International Committee on Bacteriological Nomenclature. The phages used were obtained from Dr. Blair in 1957, and subsequently propagated in the laboratory of the Division of Allergy and Infectious Diseases, Department of Medicine, the Johns Hopkins Hospital.

RESULTS

Staphylococci were isolated from the anterior nares-nasopharynx and/or purulent lesions of 111 of 205 children, or in 54.1 per cent of the individuals studied.

The highest incidence of colonization with staphylococci was found in the group under six months of age, while the lowest incidence was in the children between six months to two years of age (Table I). All the children under six months of age were delivered in a hospital. After two years of age the incidence rose to 61.7 per cent in the age group 6 to 14 years. The frequency with which coagulase positive staphylococci and penicillin resistant staphylococci were isolated also varied with age, and in a pattern similar to that seen for the total number of staphylococci isolated.

Coagulase positive staphylococci were isolated from a total of 25.8 per cent of the 205 children. While there was a definite decline in the frequency with which coagulase positive staphylococci were isolated in the 6 months to 2 years age group as compared to the group under 6 months, it was observed that much of this decline occurred between 5 months and 1 year of age (Table II). Twenty-five per cent of the infants in the age group from birth to 2 months had coagulase positive staphylococci and about the same percentage was found for those children 2 months to 5 months. However, only 6.6 per cent of the children between 5 months and 1 year of age were colonized with coagulase positive organisms.

Twenty per cent of the total number of staphylococci isolated were resistant to penicillin. A drop in the frequency of isolation of penicillin re-

TABLE I

Frequency of Isolation of Staphylococci, Penicillin Resistant Staphylococci, and Coagulase Positive Staphylococci

	Under 6 Months	6 months to 2 years	2 years to 6 years	6 years to 14 years	Totals
Number of children	40(100)*	54(100)	64(100)	47(100)	205(100)
Penicillin resistant staphylococci	15(37.5)	4(7.4)	12(18.7)	10(21.2)	41(20)
Coagulase positive staphylococci	8(20)	4(7.4)	24(37.5)	15(31.9)	51(25.8)
Staphylococci (total)	29(72.5)	16(29.6)	37(57.8)	29(61.7)	111(54.1)

* Number in parenthesis is per cent.

TABLE II

Frequency of Isolation of Staphylococci, Penicillin Resistant Staphylococci, and Coagulase Positive Staphylococci in Children under One Year of Age

	Birth to 2 months	2 months to 5 months	5 months to 1 year
Number of children	16(100)*	18(100)	30(100)
Penicillin resistant staphylococci	9(56.2)	6(33.3)	1(3.3)
Coagulase positive staphylococci	4(25)	4(22.2)	2(6.6)
Staphylococci (totals)	11(68.8)	14(77.8)	11(36.6)

* Number in parenthesis is per cent.

TABLE III

Frequency of Isolation of Staphylococci, Penicillin Resistant Staphylococci, Coagulase Positive Staphylococci, and Coagulase Positive-Penicillin Resistant Staphylococci in Relation to Hospital Contact and Penicillin Exposure

	Significant hospital contact and significant penicillin exposure	Significant hospital contact	Significant penicillin exposure	Neither significant hospital contact nor significant penicillin exposure
Number of children	21(100)*	35(100)	59(100)	90(100)
Coagulase positive staphylococci	8(38)	10(28.6)	14(23.7)	18(20)
Penicillin resistant staphylococci	7(33.3)	12(34.2)	10(16.9)	12(13.3)
Coagulase positive and penicillin resistant staphylococci	4(19)	6(17.1)	6(10.2)	8(8.8)
Staphylococci (totals)	14(66.6)	25(71.4)	30(50.8)	42(46.6)

* Number in parenthesis is per cent.

sistant staphylococci between the group of infants under 6 months and children 6 months to two years was seen (Table I). And again, this decline occurred largely in the group between 5 months and one year. Thus, 56 per cent of infants under 2 months, 33 per cent of children between 2 months and five months, and only 3.3 per cent of the patients between 5 months and 1 year had penicillin resistant staphylococci (Table II). The frequency of isolation of coagulase positive staphylococci and coagulase positive-penicillin resistant staphylococci was highest in those children who had both significant hospital contact and penicillin exposure (Table III). Penicillin resistant staphylococci were isolated more commonly in the group having significant hospital contact than in the children exposed to penicillin without hospital contact. Furthermore, the frequency of isolation of staphylococci that were coagulase positive or penicillin resistant was no higher in those children who had exposure to penicillin than in those who had no contact with the hospital nor exposure to penicillin, indicating that the penicillin exposure had no apparent effect upon coloni-

TABLE IV

Incidence of Antibiotic Resistant Strains of Staphylococci*

	Numbers of strains	Number of resistant strains						
		P	C	E	A	T	S	K
Coagulase positive staphylococci	53	26	3	1	1	3	8	1
Penicillin resistant staphylococci	46	46	4	3	0	11	11	1
Staphylococci (totals)	126	46	9	4	2	21	18	1

*Drug	Abbreviation	Concentration
Penicillin	P	7 units
Chloramphenicol	C	5 micrograms
Erythromycin	E	2 micrograms
Novobiocin	A	5 micrograms
Tetracycline	T	5 micrograms
Streptomycin	S	10 micrograms
Kanamycin	K	30 micrograms

zation with these staphylococci in children free of significant hospital contact.

Of 126 strains of staphylococci isolated, 46 (36.5 per cent) were resistant to penicillin (Table IV). Of 53 coagulase positive strains, 26 (49 per cent) were resistant to penicillin. The coagulase positive and coagulase negative strains were almost uniformly sensitive to chloramphenicol, erythromycin, and novobiocin.

The 53 coagulase positive strains were subjected to bacteriophage typing and no correlation between bacteriophage type, age of the children, or penicillin exposure could be made. Type 80/81 was isolated from three children and was found only in the anterior nares, but in each instance was found in a child with a high degree of hospital contact.

Cultures of purulent lesions were obtained from 16 patients having either conjunctivitis, otitis media, furunculosis, paronychia, other skin lesions, or an infected lymphagioma and umbilicus. From 14 of these infections a staphylococcus was isolated but in only 5 instances was the organism coagulase positive, and each of these was penicillin sensitive. Three of these five persons had significant hospital contact and two did not.

DISCUSSION

This study confirms the observations of others (6) that the frequency with which children have nasal colonization by staphylococci varies with age. In addition, these data emphasize that contact with the hospital environment affects the frequency with which children are colonized by staphylococci, penicillin resistant or otherwise.

There are several factors which should be considered as an explanation of the relation between age and colonization with staphylococci. It might

be that the variation in frequency of colonization with age simply reflects the degree of hospital contact. That is, that the high incidence of nasal colonization in newborns simply reflects colonization which occurs in hospital nurseries. This is suggested by the finding that the highest frequency of colonization was seen in children under 5 months of age with a rather marked decline between that age and one year, and by the observation that carrier rates are lower for infants born at home as compared to those born in hospitals (1). If this is part of the explanation, the factors in the hospital affecting the frequency of colonization with staphylococci may vary from one hospital to another. Epidemics of staphylococcal infections in newborn nurseries have demonstrated that no single factor (infected personnel, personnel who are carriers, wearing of masks etc.) is always important in the spread of staphylococci. Therefore, the types of organisms, staphylococci or others, with which newborns are colonized in nurseries may vary from time to time in a single hospital and from one hospital to another, depending on the source and mode of transmission of the bacteria.

If the high rate of colonization with staphylococci in infants is due to hospital contact then what is the explanation for the decline in rate after five months of age and the general rise in rate after 2 years? Cunliffe (6) attempted to relate these trends to serum levels of anti-alpha hemolysin in the patients. However, this was not successful, and at present the immune state will not explain the age variation in colonization with staphylococci. Lepper and others (11) have shown that adult hospital personnel have a high rate of "turn-over" of staphylococci in their anterior nares, and that approximately seventy-five per cent of adults change bacteriophage types of staphylococci within a month's time. If this process involves losing one strain and acquiring a new one, and if these studies are applicable to children as well as adult hospital personnel, this may explain in part the age variation observed in children. After discharge from the hospital a large number of infants spontaneously lose the carrier state and with minimal exposure to new staphylococci, because of their relative lack of close contact with anyone other than their parents, they are less likely to acquire new strains. However, sometime after two years of age, as the child becomes more gregarious, his opportunity to acquire new strains from carriers or infected persons is increased. Another possibility is that anatomical changes in the lymphoid tissue of children may alter the ability to become colonized with staphylococci. Lymphoid tissue is said to be minimal in amount at birth and shortly thereafter, but rapid hypertrophy occurs in early childhood and reaches its maximum between 6 years of age and puberty (12). There is no other evidence, however, to suggest that this is an important factor in the age variation of colonization with staphylococci.

The hospital serves as a source of staphylococci in two ways. The most

obvious is that of infected persons. Staphylococcal disease is a common type of infection in otherwise healthy individuals and it is now recognized as a common type of hospital acquired infection. Since some of the former group are admitted to the hospital and most of the latter group are already in the hospital, a concentrated source of staphylococci which may contaminate the environment either directly or indirectly is present in the hospital. In addition to this, and perhaps because of it, hospital personnel experience an increased frequency of nasal colonization with staphylococci and most of the increase is due to antibiotic resistant organisms (2). This then is an additional source of environmental "contamination" with staphylococci. Ideally, the newborn nursery is quite separate from this contaminated environment but the numerous epidemics of staphylococcal infection in these units testify to the failure of the isolation techniques used.

This study indicates that treatment with penicillin in the absence of significant hospital contact does not influence the frequency of colonization with staphylococci. It has been shown that hospitalized patients receiving penicillin acquire penicillin resistant strains more rapidly than comparable patients not receiving any antibiotics (13). This is presumably due to the fact that antibiotic therapy may remove all sensitive staphylococci and then permit the patient to acquire a penicillin resistant strain. In a non-hospital environment there are relatively fewer penicillin resistant strains present and most individuals in such a situation, therefore, may acquire penicillin sensitive organisms. The factors responsible in the hospitals for increasing the frequency of colonization have been discussed before, and it is probable that the absence of these factors in the general population explains why persons receiving penicillin outside a hospital environment do not experience an increased frequency of colonization. However, the fact that "hospital strains" of staphylococci are now distributed among the general population is well established (13, 14, 15) and it is possible that at some future date, penicillin resistant strains may be a serious problem outside of the hospital.

There were only five coagulase positive staphylococci isolated from infections during this study, and although three of the infected patients had significant hospital contact all the organisms were penicillin sensitive. This number is too small to be significant but experience with the antibiotic pattern in other patients with hospital acquired staphylococcal infections would suggest that infection attributed to penicillin sensitive strains is the exception rather than the rule (13, 15).

CONCLUSIONS

1. The frequency with which staphylococci may be found in the nares of children in a pediatric out-patient population varies with age. The

highest occurrence is in the newborns and infants under six months of age. The lowest frequency is in the age group six months to two years and after this age there is a gradual rise in the frequency through the first decade. The factors responsible for this variation are not known.

2. The majority of children from whom a staphylococcus was isolated had a penicillin sensitive, coagulase negative strain.

3. The frequency of isolation of penicillin resistant strains is related to degree of hospital contact and not to previous penicillin therapy. The highest incidence of penicillin resistant strains is found in children with significant hospital contact.

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OCCURRENCE OF HYPERTENSIVE TOXEMIA OF PREGNANCY IN MOTHER-DAUGHTER PAIRS[1]

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It is the purpose of this paper to report results of a study of the possible hereditary nature of hypertensive toxemia of pregnancy. Preeclamptic toxemia of pregnancy has long been a recognized entity and although intelligent research has attempted to disclose the basic nature of the disorder, obscurity persists. Several investigators have approached the question of whether heredity may be involved (1, 2). As is discussed later, results have been conflicting. Others have reported individual families in which several members have experienced hypertensive toxemia (3 to 6). The cause of hypertensive toxemia may well be multifactorial and many questions about pathogenesis are likely to remain after a genetic factor has been demonstrated. However, evaluation of the role of heredity is important both in the clinical management of patients and in the orientation of research.

More is known of the hereditary implications of so-called essential hypertension. Though areas of disagreement exist the evidence for familial aggregation in essential hypertension is good (7 to 14).

In this study the problem of hypertensive toxemia of pregnancy was approached by an analysis of the concordance and discordance for this trait in mother-daughter pairs. Essentially, this study determined the frequency of hypertensive toxemia in mothers grouped according to whether the daughter did or did not have hypertensive toxemia.

METHOD

The groups studied are from the case history files of the Johns Hopkins Hospital. A group of 100 patients with preeclampsia who were delivered at this hospital between the years *1945 and 1954* (henceforth referred to as the probands) are compared with a group of 200 patients with a normal pregnancy (henceforth referred to as the controls) who were delivered at this hospital between the years *1949 and 1954*. The necessity to allow for one generation and the limitations of indexing dictated the span of years from which the proband and control cases were selected. The cases were so selected that all 300 of these women were themselves born either in the Johns Hopkins Hospital or in the attendance of this hospital's then-existing Outside Obstetrical Service. Only persons with dates of birth in the year 1920 or later were selected; routine blood pressure recordings during the entire course of every pregnancy became the

[1] This work was done, in part, as a Fellow of the National Heart Institute, H.F.-7116, 1957.

TABLE I

Characteristics of the Group

	Daughters		Mothers	
	Probands	Controls	Probands	Controls
Mean Age	17.9 yrs	20.5 yrs	22.7 yrs	23.5 yrs
Primiparae	77(77%)	77(38.5%)	40(40%)	59(29.5%)
Negroes	91(91%)	161(80.5%)		
History on Microfilm			23(23%)	54(27%)

practice in 1920. All members of both the proband and the control group were born between 1920 and 1939. Because of the mode of selection, complete obstetrical data including blood pressure determinations were available for both the birth and the delivery of the selected cases. No other factors influenced the selection of cases. Further search for probands beyond the 100th case was not attempted for mathematical convenience. The control group was increased to 200 in order to include a larger number of primiparae than would be provided by studying 100 normal pregnancies.

Table I provides a comparison of the two groups with respect to age, race, parity and source of the history (histories prior to 1937 and inactive as of 1944 are stored on microfilm). The difference in the number of primiparae among the probands as compared with the controls is to be expected since preeclampsia occurs much more frequently during the first pregnancy (15). The slightly smaller number of Negroes in the control group probably represents elimination from the control group of patients with other complications of pregnancy (including prematurity) and of patients without either ante- or postpartum care at the Johns Hopkins Hospital, both of which occur more frequently in the colored race (16–19). However, a comparison of only the smaller groups of primiparae and Negroes, to be presented later, leads to the same conclusion as that of the total groups. The fact that about the same proportion of histories of the mothers of probands and controls was on microfilm suggests that the proportion of active cases was about the same in the two groups.

Acute toxemia was defined as the development, after the 24th week of pregnancy, of a diastolic pressure of 90 mm. Hg or above. As implied by the word *develop*, patients considered to have acute toxemia were demonstrated to have "normal" blood pressures prior to the 24th week and/or after the termination of the pregnancy. The elevated blood pressure had to be manifest on two occasions twenty-four or more hours apart. The control cases were determined to have a normal pregnancy by the presence of a "normal" blood pressure and the absence of any other known disease or disorder complicating the pregnancy. The systolic pressure was not used to determine the presence of toxemia because of the frequent variation of this observation with exogenous stimuli.

In the mothers of the two groups of daughters, similar criteria were used for determining the presence of acute preeclampsia; also included were those patients with acute toxemia superimposed on chronic hypertensive vascular disease as determined by the development in a chronically hypertensive patient (one with elevated blood pressure prior to the 24th week and after the cessation of pregnancy) of further elevation of the diastolic pressure by 20 mm. Hg or more. Actually the latter situation was encountered only six times, once in the proband mother group and five times in the larger control mother group.

The total proband group was divided into a) those patients in whom a diastolic

pressure of 110 mm. Hg or more was recorded during the course of the toxemia and b) those patients in whom all recorded diastolic blood pressures were less than 110 mm. Hg. No further division of the proband group into mild and severe preeclampsia was attempted.

In the follow-up study of the mothers the criteria for the presence of chronic hypertension were arbitrary. Comparisons were made of the frequency of a diastolic pressure of 110 mm. Hg (or more) 10 and 20 years following the index pregnancy. The follow-up material was heterogeneous since the figures used were those blood pressures recorded in the unit history when the patient was seen at later times in any of the departments or clinics of the hospital and for any reason. Thus the blood pressures were taken by a variety of physicians when the patient was suffering from a variety of disorders, including acute trauma. However, these qualifications presumably apply equally to the two groups of mothers.

RESULTS

A summary of the findings of the total groups is presented in Table II. As can be seen the total number of probands born of toxemic deliveries, 28 (28%), is significantly greater ($p < 0.01$) than that for the control group, 26 (13%). Furthermore, the frequency, 28 per cent, in the proband group is much greater than the general frequency of toxemia of pregnancy, about 10 to 13 per cent, observed in the Johns Hopkins Hospital in the years 1920–1939 (20, 21). The figure of 13 per cent in the control group is consistent with the observed figure of 10 to 13 per cent.

Table III presents the data on only the primiparae of each group. Here, due to the smaller groups, the significance is not so great ($p < 0.05$). Table IIIB presents the data on just the multiparae in each group. In Table IV the proband group has been divided into cases with diastolic pressures of 110 mm. Hg or more and cases with diastolic pressures of 90 mm. Hg to 110 mm. Hg. Table VA shows the data when just the Negroes in the study are compared. The percentage of Negro probands being born of toxemic deliveries is again significantly greater than that among the Negro controls. The smaller group of Negro primiparae are presented in Table VB. The very small group of Caucasian primiparae is presented in Table VI. If only the probands born of primiparous pregnancies are compared with the controls born of primiparous pregnancies similar differences are found, as indicated in Table VII.

The differences between the proband and control groups presented in

TABLE II

Total Group

	Number in Group	Frequency of Toxemia in Mothers
Mothers with Toxemic Daughters	100	28(28%)
Mothers with Non-Toxemic Daughters	200	26(13%)

TABLE IIIA

Total Primiparae

	Number in Group	Frequency of Toxemia in Mothers
Mothers with Toxemic Daughters	77	22(28.7%)
Mothers with Non-Toxemic Daughters	77	11(14.3%)

TABLE IIIB

Total Multiparae

	Number in Group	Frequency of Toxemia in Mothers
Mothers with Toxemic Daughters	23	6(26.1%)
Mothers with Non-Toxemic Daughters	123	15(12.2%)

TABLE IV

"Severity" of Toxemia

(as determined by the diastolic blood pressure)

	Number in Group	Frequency of Toxemia in Mothers
Probands with Diastolic > 110 mm. Hg	51	17(33.3%)
Probands with Diastolic < 110 mm. Hg	49	11(22.4%)
Controls	200	26(13%)

TABLE VA

Total Negro Patients

	Number in Group	Frequency of Toxemia in Mothers
Mothers with Toxemic Daughters	91	25(27.5%)
Mothers with Non-Toxemic Daughters	161	20(12.4%)

TABLE VB

Negro Primiparae

	Number in Group	Frequency of Toxemia in Mothers
Mothers with Toxemic Daughters	69	19(27.5%)
Mothers with Non-Toxemic Daughters	62	8(12.9%)

Tables III through VII are not statistically significant; the numbers are smaller in each of these sub-groups. However, the striking consistency of the differences in the sub-groups increases our confidence in the differences obtained in the total group, as presented in Table II.

TABLE VI

White Primiparae

	Number in Group	Frequency of Toxemia in Mothers
Mothers with Toxemic Daughters	8	3(37.5%)
Mothers with Non-Toxemic Daughters	15	2(13.3%)

TABLE VII

Parity of the Mothers

	Born of Primiparous Pregnancies	Frequency of Primiparity and Toxemia in Mothers
Mothers with Toxemic Daughters	40	13(30.8%)
Mothers with Non-Toxemic Daughters	59	9(15.4%)

The results of the ten and twenty year follow-up of the maternal group suggest that a definitely elevated blood pressure develops more frequently in the mothers of the probands than in the mothers of the controls. However, the difference between the two groups is not statistically significant in these small numbers.

DISCUSSION

The fetal mortality rate in toxemic pregnancies is higher than for the general population (22). However, because of the mode of selection in this study, the results would probably not be influenced by this fact.

A bias present in some studies—that the presence of a given disorder in one member of the family leads to more thorough search for the disease in other members of that family than is the case in control families—was not operative in this study because of its particular design.

The results of this study would appear to contradict the earlier study of Barnes and Browne (1) who found no increased frequency of hypertension among relatives of toxemic patients. However, they are consistent with the later findings of Browne and Sheumack (2) which led to the conclusion that toxemia of pregnancy might be the early expression of the hypertensive tendency which exists in the family. This conclusion was based on the follow-up of toxemic patients whose relatives exhibited hypertension and comparing these with toxemic patients whose relatives were not hypertensive. Also the results of the present study agree with the conclusions of Tillman and Herrick, Eastman, and Plass stated at the Conference on Eclampsia in 1941 and summarized by Dieckmann: "that a much greater incidence of toxemia occurred in patients with a family history of vascular disease than in the control group" (23).

SUMMARY AND CONCLUSIONS

One hundred patients who demonstrated hypertensive toxemia of pregnancy in the Johns Hopkins Hospital between the years 1945 and 1954 (probands) and were themselves born on the Obstetrical Service of this hospital were compared with two hundred patients with normal pregnancies delivered in this hospital in the years 1949 and 1954 (controls) and also born in this hospital. The two groups were similar with respect to age and race. It is noted that 28 (28%) of the probands were themselves born of a toxemic pregnancy whereas only 26 (13%) of the control group were born of such pregnancies. During the involved period the general frequency of toxemia of pregnancy in this hospital was about 12 per cent.

A follow-up study of the mothers 10 and 20 years after the index delivery revealed that elevated blood pressures were more prevalent among the mothers of the probands than among the mothers of the control group.

It is concluded that hypertensive toxemia of pregnancy displays familial aggregation and that it is one expression of a familial, presumably inherited, tendency toward hypertensive disease.

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INTERACTION OF SERUM AND SODIUM SALICYLATE: CHANGES DURING ACUTE INFECTION AND ITS INFLUENCE ON PHARMACOLOGICAL ACTIVITY[1]

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Pharmacological agents vary in their affinity for plasma protein, depending on the size, configuration, polarity, solubility, and other physical properties of the drug molecule. In addition, the firmness of the drug-protein bond is variable, suggesting that there are different mechanisms of binding. Coulomb forces, Van der Waal forces, adsorption, and hydrogen bonds are probably all implicated in the interaction of drugs with protein (1). Virtually all drugs and simple chemicals combine with plasma albumin, whereas many cations, hormones, vitamins, and endogenously produced substances (steroids, hemoglobin, etc.) preferentially unite with alpha or beta globulins (1). With some agents (Evans blue dye) there are a finite number of molecules capable of combining with albumin, but probably in all instances, the amount of drug bound to protein varies with the concentration of the two reactants (drug and protein) (2).

Ultrafiltration and equilibrium dialysis are the two principle methods that have been used for studying drug-protein interaction. Occasionally, a drug can be shown to move electrophoretically with a specific protein but this indicates binding only if the drug alone has a different electrophoretic mobility than the drug-protein complex. Penicillin and human albumin may move at identical rates in zone electrophoresis, and this fails to differentiate the bound and unbound drug (3). In some instances, the combination with protein will alter the absorption spectrum of a drug (1).

Various investigators have shown that antibacterial agents bound to protein may be pharmacologically inactive (4, 5). Changes in drug-protein binding may alter not only pharmacological activity, but may be expected to change rates of metabolism and urinary excretion. The pharmacological response of an individual to a drug is usually predictable, but occasionally is exaggerated (*drug idiosyncrasy*), and it is possible that this hyperreactivity of some individuals depends on changes in drug-protein binding.

Landsteiner (6) showed conclusively that a simple chemical was able to induce hypersensitivity in animals only if first united to a protein. From

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his experiments it was suggested that drug allergy may first involve an union between the drug and some body protein. Evidence for an unique drug-protein interaction in drug hypersensitive individuals, however, has not been demonstrated.

Chabanier, Lebert, and Lobo-Onell (7, 8), showed that salicylate is bound to serum proteins *in vivo* and *in vitro*. Storm van Leeuwen (9) reported a decrease in serum protein binding of salicylate in individuals with asthma and aspirin hypersensitivity. A marked decrease in salicylate binding has also been noted in some rheumatic fever patients (10). No studies are known to show variations in pharmacological activity of salicylate dependent upon changes in binding to protein.

The present study was undertaken to review the salicylate-protein binding characteristics of human sera, to determine if there were variations in serum protein binding in indiviuals with aspirin hypersensitivity, and to see if changes in the amount of salicylate bound to serum protein could possibly alter the drug's pharmacological activity.

MATERIALS AND METHODS

Serum: Serum was obtained from normal, acutely ill, asthmatic, and aspirin hypersensitive individuals. The blood was collected by venipuncture with aseptic precautions, allowed to clot at room temperature, and the serum was stored at 4°C. Rabbit blood was obtained by cardiac puncture and the serum separated and stored in the same way.

Serum Protein Fractions: Lyophilized Cohn fractions V, IV 1, and IV 4 were supplied by the American Red Cross and dissolved in 0.85 per cent NaCl in various concentrations. Gamma globulin was obtained by ammonium sulfate fractionation. The protein fractions were characterized by paper electrophoresis (11).

Salicylate: Sodium salicylate was dissolved in distilled water in concentrations of 1, 2, and 4 mg. per ml. These were stored at 4°C. Aliquots of the stock solutions were diluted with saline prior to use. The pH values of salicylate-saline solution were 6.8 to 7.0.

Bacterial Pyrogen: A purified protein-lipo-polysaccharide complex from Shigella flexneri (12) was used as a pyrogen in a concentration of .01 microgm. per ml. and injected intravenously into rabbits.

Measurement of Temperature: The febrile response of rabbits was measured by an indwelling rectal resistance coil and recorded by a Telethermometer, as described elsewhere (13).

Dialyses: Sodium salicylate was prepared in concentrations of 25, 50, 100, and 200 microgm. per ml. as described above. Five ml. of serum or purified serum protein were placed inside cellophane tubing and dialyzed against the various concentrations of salicylate at 4°C. for 48 hours. After dialysis, salicylate determinations were performed on the dialysant and dialysate.

Chemical Measurements: The method of Brodie, Udenfriend, and Coburn was used (14) to determine salicylate concentrations. A biuret method was used to determine total serum protein (15).

Electrophoresis: Human and rabbit sera were analyzed for protein composition by

paper electrophoresis (11) carried out in a Spinco-Durrum type cell. One one-hundredth of a milliliter of serum was placed on 3 × 32 cm. Whatman No. 3 filter paper strips; 0.075 M Na veronal-veronal buffer at pH 8.6 was used and the procedure carried out for 16 hours at 5 ma. Stained strips were scanned photoelectrically in a Spinco-Model R Analytrol and fractions computed in per cent of total protein.

EXPERIMENTAL RESULTS

Salicylate binding of normal human sera: Dialysis and ultrafiltration experiments have shown significant binding of salicylate to proteins of human sera. Chabanier, Lebert, and Lobo-Onell in 1923 (7) showed that salicylate bound to protein would not pass through a semi-permeable membrane. Serum dialysed against isotonic saline containing salicylate quickly accumulated salicylate in greater concentrations than in the saline solution. In 1946, Smith *et al.* (16) studied by untrafiltration the protein binding of serum from patients receiving salicylate. It was found that with serum salicylate concentrations of 200 mg. per liter less than one-fourth was unbound, but at 500 mg. per liter almost one-half was unbound. No variations in binding from pH 7.4 to 8.1 were noted. Lester, Lolli, and Greenberg (10) used both dialysis and ultrafiltration to study drug protein binding and also found that the proportion of bound salicylate decreases with increasing concentration of salicylate. Temperatures of 6°C. to 37°C. did not appreciably affect the degree of binding. To reinvestigate salicylate binding by normal human sera, the following experiments were performed.

Salicylate solutions were diluted with 0.85 per cent NaCl to make concentrations of 25, 50, 100, and 200 microgm. per ml. Five ml. aliquots of normal human serum in cellophane tubing were dialyzed against 50 ml. of each of these solutions. Dialysis was carried out for 48 hours at 4°C. Salicylate determinations of the serum were then performed. Values did not change if the dialysis was extended beyond 48 hours.

The salicylate binding of 8 different normal human sera are plotted in Figure 1. It was apparent that the serum contained higher concentrations of salicylate than the dialysate, indicating binding of drug to serum protein. Data in Figure 1 also revealed increased binding of salicylate as the drug concentration was increased, but the per cent of total drug bound to protein decreased. These results are in complete accord with the investigation described earlier.

Van Leeuwen and Drizmal (9) reported that serum from persons with asthma bound salicylate less actively than serum from normal individuals. Lester *et al.* (10) found less binding of salicylate by sera from rheumatic fever patients. They reported one case where only 6 per cent of the total salicylate was bound when the plasma drug concentration was 37 mg. per cent.

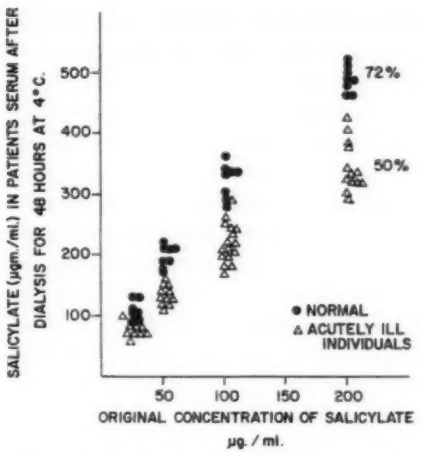

FIG. 1. The binding of salicylate by serum from normal and acutely ill individuals. Per cent binding calculated on the basis of the difference between the concentration of salicylate in the dialysant and dialysate after dialysis.

TABLE I

Case Summaries—Acutely Ill Patients

Pt.	Age	Race	Sex	
C.M.	40	C	F	Lung abscess—bacteroides
J.T.	67	C	M	Klebsiella pneumonia, chronic bronchitis and emphysema
A.C.	40	C	F	Acute endocarditis, left hemiparesis; died
B.C.	14	C	M	Furuncle, staphylococcal septicemia and pneumonia
V.E.	24	C	F	Infectious mononucleosis
E.M.	21	C	F	Rheumatic heart disease—3 days post partum developed gram neg. septicemia and died
O.M.	40	W	F	Unresolved RML & RLL pneumonia with pleural effusion
C.M.	23	C	F	Grand mal epilepsy; lung abscess and empyema—bacteroides
S.K.	27	C	F	Gonococcal arthritis
G.W.	41	W	M	Pneumococcal pneumonia; lung abscess—bacteroides
S.C.	14	C	M	Acute rheumatic fever
G.P.	45	W	F	Tuberculosis, pulmonary

To study possible variations in salicylate binding by sera from sick patients, blood was obtained from several persons with acute, severe infection. The nature of the infections are briefly summarized in Table I.

The sera from patients with acute infection were dialyzed against increasing salicylate concentrations in 0.85 per cent NaCl as described in the preceding section. The binding of salicylate by the sera from the acutely ill patients, as determined by equilibrium dialysis, is shown in

Figure 1. The binding activity of this group of sera for salicylate was significantly less than the activity of normal sera.

Sera from six patients with long standing histories of chronic asthma were similarly studied for their ability to bind salicylate. In every instance, the salicylate binding characteristics of these sera did not differ from that of normal sera.

From these experiments it was clear that decreased salicylate binding by plasma protein occurred during acute infection. There was no indication that sera from patients with asthma, free of acute infection, had a reduced salicylate binding activity.

Protein Fractions and Salicylate Binding: Virtually all drugs which bind to plasma protein interact with albumin. The following studies were done to characterize the protein fraction that combined with salicylate.

Cohn's fraction V, IV 1 and IV 4 in varying concentrations were dissolved in 0.85 per cent NaCl. By electrophoresis solutions of fraction V revealed almost pure albumin. Fraction IV 1 and IV 4 were a mixture of alpha and beta globulins. Five ml. of the protein solutions were dialyzed against 50 ml. of salicylate as described above.

Fraction IV 4 and IV 1 revealed equal quantities of salicylate in the dialysant and dialysate, indicating no binding to these serum proteins. Fraction V showed considerable interaction with salicylate, the degree of binding closely resembling that of whole human serum (Fig. 2). Gamma globulin was prepared from normal human sera by ammonium sulfate precipitation and reconstituted to 3 gms. per cent. Dialysis of the gamma globulin against salicylate showed no detectable binding by the protein fraction.

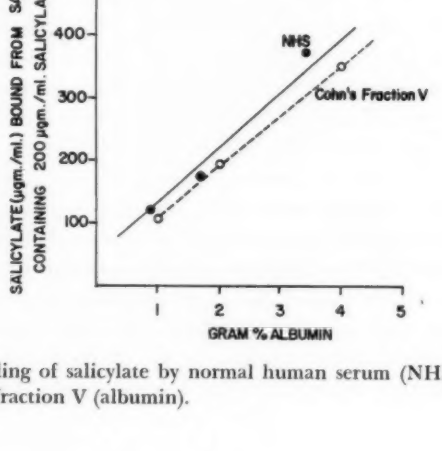

FIG. 2. The binding of salicylate by normal human serum (NHS) compared to the binding by Cohn's fraction V (albumin).

From these *in vitro* studies it seemed clear that only albumin was capable of interacting with salicylate to form a drug-protein complex.

Aspirin Hypersensitivity and Salicylate Binding: A qualitative or quantitative difference in drug serum protein interaction might be present in drug hypersensitivity, as has been described before (17, 18, 19). Storm Van Leeuwen (20) reported decreased salicylate binding in aspirin hypersensitive individuals and even suggested that this might in some way be correlated with drug hypersensitivity.

The salicylate binding characteristics of the serum from three aspirin hypersensitive individuals were studied. Two of these individuals responded to the ingestion of aspirin with acute attacks of asthma, one with both asthma and hives.

By equilibrium dialysis, experiments similar to those described above were performed. No differences in the salicylate binding pattern of hypersensitive and normal sera were demonstrated. Gamma globulin was separated by ammonium sulfate precipitation from the sera of two persons with aspirin hypersensitivity. This globulin was reconstituted to normal serum concentration. Salicylate binding by the globulin fractions of serum was studied by means of equilibrium dialysis. No drug combined with gamma globulin derived from these hypersensitive individuals' sera.

By the methods used in these experiments there was no detectable difference between salicylate binding by normal serum and serum from aspirin hypersensitive persons.

Effect of Varying Albumin Concentration on Salicylate Binding: Previous investigators have shown reduced salicylate binding with decreased levels of plasma albumin. The following experiment was designed to show the influence of albumin concentration on the protein binding of salicylate.

Five ml. of 4 gm. per cent Cohn's fraction V and undiluted normal human serum and dilutions of 1:2 and 1:4 of each were dialyzed against 50 ml. of 0.85 per cent NaCl containing salicylate in a concentration of 200 microgm. per ml. The salicylate concentrations of the serum and fraction V solutions were determined after dialysis as described before.

A direct relationship between the quantity of salicylate bound to protein and the amount of albumin present for both Cohn's fraction V and normal human serum was shown (Fig. 2).

Albumin Concentration of Acutely Ill Patients and Salicylate Binding: In the above experiments reduction in albumin concentration decreased the protein binding of salicylate. To determine if changes in serum albumin concentration could account for the reduction in protein binding of salicylate in the acutely ill patients the following study was performed.

Sera from normal individuals and the patients with acute infection were examined by paper electrophoresis. Total protein of the sera was deter-

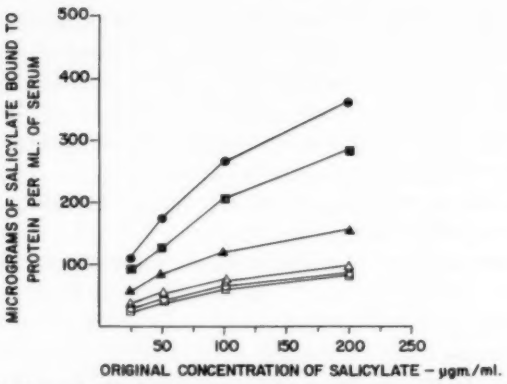

FIG. 3. The binding of salicylate per ml. of serum from normal (●), acutely ill (▲), and convalescent (■) individuals, compared with the expected binding capacity of each gm. per cent of albumin per ml. of serum from the same normal (○), acutely ill (△), and convalescent (□) individuals.

mined by the biuret method. The concentration of albumin was calculated from the electrophoretic curve and the total serum protein.

The salicylate protein binding of a normal, convalescent, and an acutely ill patient is illustrated in Figure 3 and compared with the predicted drug binding activity of the sera per gm. of albumin. It was apparent that the changes in binding capacity of the sera were readily accounted for by the differences in albumin concentration.

Drug Binding, Albumin Concentration, and Pharmacological Activity: Studies with other drugs, particularly with antibacterial agents, have correlated pharmacological activity with the concentration of unbound drug in serum; the drug-protein complex being inactive. The experiments reported here have shown that acutely ill patients may have lowered plasma albumin concentration resulting in a decrease in salicylate binding activity of the serum. To determine if changes in serum albumin concentration could influence the pharmacological activity of salicylate the following experiment was done.

The concentration of plasma albumin was raised in a group of 6 normal rabbits by injecting intravenously 10 gm. of Cohn's fraction V. Twenty-four hours later the group injected with fraction V and a second group of 6 normal rabbits were given the bacterial pyrogen intravenously and temperatures were recorded for 6 hours. In a similar experiment, one group of rabbits was given fraction V and 24 hours later the two groups of animals were given 0.5 gm. of sodium salicylate intravenously, 30 min. before injection of the pyrogen.

The serum albumin concentration in the rabbits 24 hours after injection

TABLE II

Albumin Concentrations and Salicylate Binding Activity of Serum from Normal and Hyperalbuminemic Rabbits

Serum	Albumin Concentration	Salicylate Bound
Normal Rabbits (3 animals)	3.7 gm. per cent	150 microgm. per ml.
Rabbits 24 hours after 10 gm. fraction V I.V. (3 animals)	6.9 gm. per cent	270 microgm. per ml.

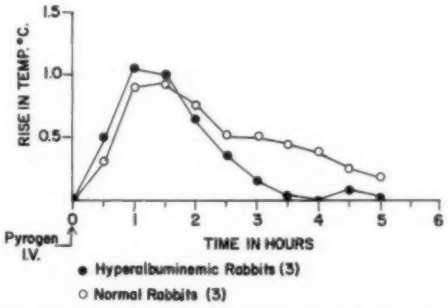

FIG. 4. The febrile response to 0.01 microgm. bacterial pyrogen (I.V.) of normal rabbits and rabbits given 10 gm. of fraction V (I.V.) 24 hours previously (hyperalbuminemic).

of fraction V was nearly twice that of the normal animals. In addition, the salicylate binding activity of the sera of the rabbits given fraction V was twice that of normal serum Table II. The febrile response to pyrogen of the hyperalbuminemic animals was no different than that of normal rabbits (Fig. 4). However, the two groups of animals (normal and hyperalbuminemic) given salicylate 30 min. before injection of bacterial pyrogen had differing febrile responses (Fig. 5). Administration of salicylate to the rabbits with increased serum albumin had less antipyretic effect than the same dose of salicylate given to normal animals.

From these experiments it appeared that the antipyretic activity of salicylate had been reduced or eliminated by hyperalbuminemia, associated with an increased binding activity of the serum for salicylate.

DISCUSSION

When salicylate is added to normal human serum, the majority of drug is bound to albumin. The extent of the binding will depend on the dose of the salicylate and the concentration of albumin. The exact nature of the bond is unknown but some recent work by Davidson *et al.* (21) on the binding of simple benzene derivatives, including salicylate, by bovine serum albumin reveals two types of interaction. One of the drug protein

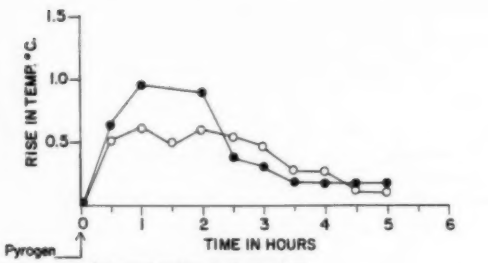

FIG. 5. The febrile response of normal rabbits and hyperalbuminemic rabbits (see Fig. 4) given 0.01 microgm. bacterial pyrogen 30 minutes after receiving 0.5 gm. Na salicylate I.V.

bonds is readily dissociable while the other is not. The relationship between free and bound salicylate is easily altered by changing the concentrations of reactants, drug and albumin, but equilibrium is established between the bound and unbound drug.

The experiments described here have shown a decrease in pharmacological activity of salicylate that is related to an increase in plasma albumin concentration. It is of interest that a decrease in antibacterial activity of chemotherapeutic agents has been attributed to increased protein binding (5). Fifty to 60 per cent of penicillins G, X, and F are bound to plasma albumin but 90 per cent of penicillin K is similarly bound. Penicillin K has less antibacterial activity *in vivo* than penicillins G, X, and F, and this has been related to its increased protein binding. In contrast penicillin K in the absence of serum albumin has slightly greater antibacterial activity than penicillins G, X, and F. Davis (22) has shown a wide variation in the binding of sulfonamides to plasma albumin. Again the antibacterial property of the sulfonamides was correlated with the level of unbound drug in the plasma. Serum albumin added to culture media containing fatty acids, permits the initiation of growth of tubercle bacilli. Davis and Dubos (23) showed that the role of albumin was to bind traces of oleic acid, and that the unbound oleic acid prevented the growth of tubercle bacilli. The failure of oleic acid as an antituberculous agent was related to its inactivation by combination with serum albumin.

There seems little doubt that many drugs bound to serum protein are pharmacologically inactive. Other evidence suggests that endogenous substances united to proteins are biologically inactive. Calcium is present in plasma in a concentration of about 10 mg. per cent. Half of this is bound

to proteins, both albumin and globulin, the remaining being in solution as either calcium ion or calcium salts (24). The unbound calcium is freely diffusible and accounts for the biological activity of the cation. If the plasma proteins are lowered, the total calcium concentration of plasma is reduced, but this reflects only a reduction in the protein bound calcium. The diffusible calcium remains approximately normal and manifestations of hypocalcemia do not develop (24). Recently it has been shown (25) that a clotting abnormality, an abnormal thrombin time, seen in some patients with hyperglobulinemia, is attributable to a reduction in ultrafiltrable calcium associated with the binding of calcium to the increased globulin. Correction of this clotting defect *in vitro* and *in vivo* was accomplished by the administration of calcium ions.

The pharmacological activity of salicylate is directly related to the quantity of drug that is not bound by plasma albumin. By increasing the plasma albumin concentration of rabbits, the antipyretic activity of salicylate was lowered. These animals had higher levels of both plasma albumin and albumin-bound salicylate.

There are many clinical situations where plasma albumin concentration is greatly reduced, such as in infection (26). In the experiments reported here it has been shown that reduction in serum albumin concentration is associated with a decreased *in vitro* binding of salicylate to serum protein. The decrease in antipyretic effect of salicylate in rabbits produced by an increase in plasma albumin concentration, and the decrease in binding properties of human sera with reduced albumin concentration, suggests the possibility that an exaggerated pharmacological response may occur in those individuals with depressed serum albumin. Idiosyncratic drug reactions in some instances may be explained on this mechanism (Fig. 6). This explanation for drug idiosyncrasy would apply only to therapeutic agents which bind significantly to plasma proteins so that decreased binding associated with lowered protein concentrations would in effect make available a quantity of drug able to demonstrate increased pharmacological activity.

The pharmacological activity of any drug, it should be emphasized, is conditioned by a series of complex, integrated events, including absorption, distribution, metabolism, excretion, and responsiveness of the end-organ to the drug. The influence of protein binding upon the pharmacological activity of a drug, as already discussed, is, nevertheless, a probable explanation for changes in response to a drug in patients with quantitative alterations in plasma proteins.

The studies reported here raise the distinct possibility that drug toxicity in man may be ameliorated by the administration of the serum protein which will bind the therapeutic agent.

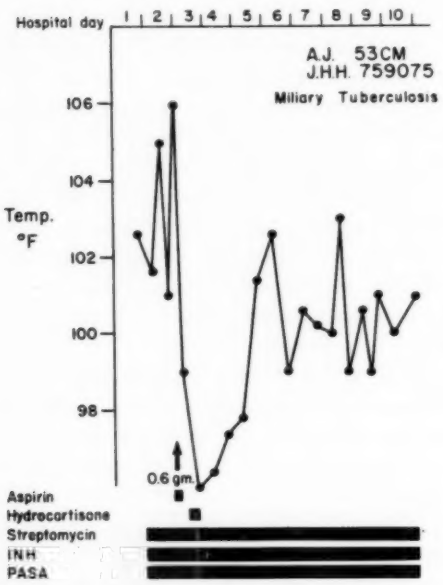

FIG. 6. The exaggerated antipyretic effect of 0.6 gm. aspirin in a patient with miliary tuberculosis. (Reprinted from *Drugs of Choice* with the approval of Walter Modell, M.D., Editor, C. V. Mosby, St. Louis, Publisher)

Serum antibodies might be expected in individuals who respond to ingestion of aspirin with urticaria, hives, or asthma. If serum antibody were present, it may bind salicylate as well as albumin. Under these circumstances the total amount of salicylate bound by hypersensitive sera would be increased. No differences, however, in salicylate binding properties of normal and hypersensitive sera were noted.

SUMMARY AND CONCLUSIONS

1. Salicylate binds only to plasma albumin.
2. Variations in the binding capacity of different sera for salicylate are entirely dependent on changes in the albumin concentration.
3. Serum from individuals with aspirin hypersensitivity demonstrates salicylate binding identical to serum from non-hypersensitive persons.
4. The pharmacological activity of salicylate is partially dependent on the magnitude of the drug-protein interaction and can be interfered with by increasing albumin concentration. In some instances drug idiosyncracy may be the result of reduced drug-protein interaction, when the binding protein is depleted by disease states.

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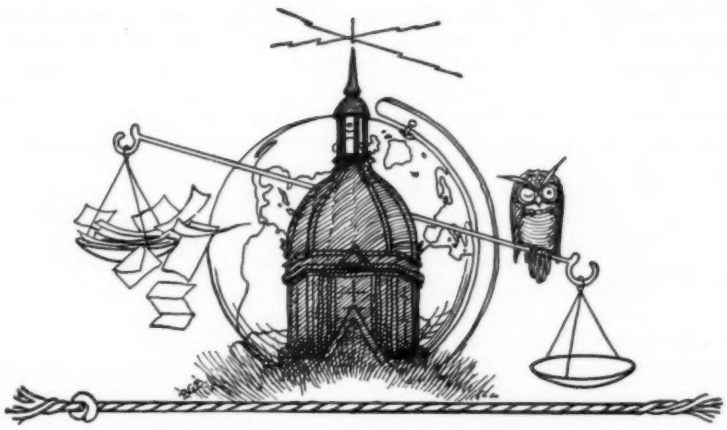

BOOK REVIEWS

Surgical Anatomy of the Bronchovascular Segments. By WILLIAM E. BLOOMER, AVERILL A. LIEBOW AND MILTON R. HALES. With a Foreword by Gustaf E. Lindskog. 273 pp., $16.50. Charles C Thomas, Publisher, Springfield, Ill., 1960.

This book is a very good detailed description of the surgical anatomy of the lungs. The terminology and nomenclature is that developed by Boyden. Undoubtedly, from an anatomical standpoint, this classification is very useful; however, it does have some limitations particularly because it is excessively detailed. Separate chapters are devoted to each lobe of the lung, and the various surgical anatomical aspects are considered. There is a final chapter which considers the radiological features of the problem, including good angiocardiographic studies. The illustrations in this text are numerous, a number are colored, and all are of excellent quality. The bibliography is limited, but contains the most notable contributions to this field. As a supplement to the illustrations there is available a number of stereoscopic transparencies which aid considerably in the visualization of the complex anatomical relationships.

This volume should prove useful as a reference text to those interested in thoracic surgery, but also would be of value to anatomists. The book is highly recommended for such purposes.

WILLIAM F. RIENHOFF III

Symposium on Salt and Water Metabolism. Edited by ALFRED P. FISHMAN. 254 pp., $2.00. American Heart Association, New York Heart Association, New York, N. Y., 1960.

The papers contained in this volume are edited versions of those given at the Symposium held in New York in December 1959. The format is that of the periodical, Circulation, in which this material was originally published as a supplement.

The subject matter covers a wide range, from the theory of solutions to purely descriptive physiological studies. Among the systems discussed are the gut, the eye, the bile, cartilage, the avian salt gland, the endocrines and, of course, the kidney. No attempt at

complete coverage was intended; the selection of material seems to have been based on recency of material and the excitement generated by it. The quality of the presentations is almost uniformly high. The discussions following some of the papers are particularly illuminating. In both papers and discussions, there is considerable speculation. While some of the attempted syntheses are premature in that they hinge on very incomplete data and tenuous assumptions, they make good reading.

The book will undoubtedly enjoy considerable attention from both physiologists and physicians.

I. M. WEINER

Henry E. Sigerist on the History of Medicine. Edited by FÉLIX MARTI-IBANEZ. 336 pp., $6.75. MD Publications, Inc., New York, 1960.

Henry E. Sigerist on the Sociology of Medicine. Edited by MILTON I. ROEHMER. 406 pp., $6.75. MD Publications, Inc., New York, 1960. The two volumes ordered as a set $10.00.

These two volumes consist, largely, of reprints of articles previously published in various journals. For instance, twenty of the fifty-eight pieces have appeared in the *Bulletin of the History of Medicine*, and its predecessor.

The book on the history of medicine groups the articles under I—On Medical History, II—Ancient and Medieval Medicine, III—Renaissance, Baroque and Age of Enlightenment Medicine, and IV—Personal History. Some of the selections are rather slight, almost ephemeral, productions—talks given for special occasions. Because of this, there is a good deal of repetition of ideas in many of the articles. The editor, of course, cannot be blamed for this, since his purpose was to compile a collection of papers and not an anthology. Another self-imposed restriction was the decision to include only material that had not appeared elsewhere in book form. This apparently meant the exclusion of some of Sigerist's most important contributions to the history of medicine, even though these had appeared first as periodical articles. It was Sigerist's custom to publish collections of his own articles, either in the form as originally published, as in *The University at the Crossroads*, or to re-work the material in articles into a different presentation as in *Civilization and Disease*. Beyond this restriction though some of Sigerist's really valuable articles are included in the present work and it is good to have them brought together in one place.

The volume on the sociology of medicine is a more complete presentation. There are articles covering all aspects of Sigerist's interest in the social aspects of medicine, and particularly of his ideas on socialized medicine. The work is divided into five sections, I—General Essays, II—Europe, III—America, IV—Other Lands, V—Special Topics. Three of the articles were previously unpublished and four are translations from French and German.

Together these two volumes give a good picture of Sigerist's many and wide interests. Since they are collections of articles, they are not books to be read through at one sitting, but rather are better suited for occasional browsing. Most of the pieces are extremely good reading, in Sigerist's delightful, informal style.

SANFORD V. LARKEY

Carter's Microbiology and Pathology, 7th edition. By ALICE L. SMITH. 742 pp., $7.50. The C. V. Mosby Co., St. Louis, Mo.

This general textbook is divided into two parts: microbiology and pathology. The

first two-thirds deals with general microbiology including bacteriology, immunology, parisitology, mycology and virology. Selected practical applications emphasize the role of useful and pathogenetic microbes in medicine, nursing, industry and public health.

The second part is a brief introduction to general and specific pathology. The importance of clinical and post-mortem pathology is well-presented; particularly good is the discussion of *why* an autopsy should be performed and *how* it may benefit medical practice and investigation. Operating room consultation and frozen section diagnosis by the surgical pathologist deserves more emphasis upon their *immediate* value to both patient and surgeon. Although prompt fixation of biopsy specimens is important for optimal histologic preparations (p. 431), the enormous importance of cultures of biopsied inflammatory lesions makes it mandatory to obtain fresh, uncontaminated fluid or tissue *prior to* fixation. The increasing value of exfoliative cytology for the diagnosis of a variety of neoplasms is not brought out in the introductory chapters on pathology and should likewise have been emphasized in the sections devoted to pulmonary, pleural, peritoneal, gastrointestinal and genito-urinary lesions. The brief mention of this valuable diagnostic technic in the chapter dealing with diseases of the female reproductive organs (pp. 646–7) is hardly in keeping with these broader applications.

The format and illustrations are of good quality and the glossary of terms and index are useful. This text is suggested for general courses in microbiology and pathology for students of nursing and related fields.

SUMNER WOOD, JR.

Heritable Disorders of Connective Tissue, 2nd edition. By VICTOR A. MCKUSICK. 333 pp., $12.00. C. V. Mosby Co., St. Louis, Mo., 1960.

Discussion in the second edition of this important book again is organized around the five disorders that Dr. McKusick believes are best explained as hereditary generalized defects of connective tissue: the Marfan syndrome, the Ehlers-Danlos syndrome, osteogenesis imperfecta, pseudoxanthoma elasticum, and the Hurler syndrome. The book again features minutely detailed clinical descriptions of these diseases. Many of the comments on rarer manifestations and associated lesions have been revised in light of the many publications that have appeared during the four years since the first edition or on the basis of personal observations by Dr. McKusick and his colleagues.

In addition to these five major disorders, numerous eponymic or "unpronounceable" malformations and diseases of familial or alleged familial occurrence are discussed, usually on the grounds that they may eventually be shown to represent localized or generalized defects of connective tissue. Whether the reader accepts or discards the probability of an underlying defect in connective tissue for any one of these in no way influences the value of this well-organized compendium of factual information on obscure and poorly understood entities.

A feature which enhances the interest and value of the book to clinicians, geneticists, biochemists, and other specialists is the consistent emphasis upon problems that remain to be solved. For each disease, a critical summary of the known, the probable, and the possible facts as they now stand is followed by a careful attempt to relate them to hypotheses of origin and mode of transmission of the disorder, to questions about genetic variants, to the basic biochemical or metabolic mechanisms that might underlie its manifestations, etc. Finally, suggestions are made about profitable lines for future study ranging from simple cataloguing of clinical features to epidemiologic, genetic, and enzymatic investigations of great complexity. The result is an extraordinarily useful guidebook for those who would study these diseases.

Dr. McKusick has traced many traditional "clinical impressions" concerning these diseases to their origins and has evaluated their worth. Some contain a core of truth but, as might be expected, many are erroneous. Nor will it come as a surprise to the reader to learn that most of the false pronouncements are attributable to that almost irresistable impulse to pad bibliographies by quoting "all of the literature" that has led (and still leads) medical writers to cite references that they have ready "by title only". For example, Dr. McKusick points out the rarity of patent ductus arteriosus in the Marfan syndrome, noting that a frequently cited case report in the French literature actually describes a patient with Marfan syndrome and "trou de Botal" (patent foramen ovale) rather than "ductus de Botal" (patent ductus arteriosus).

The book is well-illustrated and extremely easy to read. Extensive historical discussion is presented with a pertinence that guides the entire presentation and makes it meaningful rather than dominating it or giving the impression that the author is making a formal summary of past events because erudition demands it. The inclusion of interesting sidelights such as marshalling the evidence in support of Lautrec's having had osteogenesis imperfecta and pointing out that the "India rubber man" of the side-show is, in all likelihood, a victim of the Ehlers-Danlos syndrome enhance the text. Occasional touches of humor range from a short discussion of the proper pronunciation of that pesky word, SYNDROME, to half-serious regret that William the Conqueror exhumed and cremated Ivar the Boneless with the result that his skeleton is not available for study by those who conjecture that the bonelessness was osteogenesis imperfecta.

The increasing rarity of specimens of medical writing that simultaneously possess the attributes of *readability* and *scholarship* makes this book mandatory for libraries, public and private; it is informative but, more important, it restores faith.

IVAN L. BENNETT, JR.

A Synopsis of Anaesthesia, 4th edition. By J. ALFRED LEE. 616 pp., $6.50. John Wright & Sons, London. The Williams & Wilkins Co., Baltimore, Md., exclusive U. S. agents.

Although this book takes the English point of view as regards technique and drugs, it makes excellent reading for any one interested in anesthesiology. It covers the entire field of anesthesiology better than any single book extant. The format is one of brief outlines with explanations which are clear and cryptic. This latest edition has been completely revised and now includes a discussion of such agents as the phenothiazines and halothane, and such techniques as hypothermia.

As one reads this book, one is amazed by its completeness. It touches on nearly every subject within the ken of anesthesiology. It is probably for this reason that it is held in such high esteem by students and practitioners for review in preparation for examination.

DONALD W. BENSON

Diseases of Metabolism, 4th edition. Edited by GARFIELD G. DUNCAN. 1104 pp., $18.50. W. B. Saunders Co., Philadelphia, Pa., 1959.

This work has become the standard reference of the clinician for problems in metabolism. Each contributor has reviewed recent developments pertaining to his topic and has extracted clinical implications from the facts where ever possible.

In addition to thorough revision of each section, several chapters have been entirely rewritten. The scholarship of Lipid Metabolism by Gurin is particularly noteworthy. The section on Water Balance by Welt is also a lucid exposition of a difficult subject.

The chapters on Protein Metabolism, Obesity and Glycogen Storage Diseases are excellent reviews on these subjects. This volume will continue the tradition of excellence set by earlier editions.

T. E. PROUT

BOOKS RECEIVED FOR REVIEW

The Art of Marriage Counseling. A Modern Approach. By W. L. HERBERT AND F. V. JARVIS. 125 pp., $2.75. Emerson Books, New York, N. Y., 1960.

Cold Injury. Transactions of the Sixth Conference July 1958. Edited by STEVEN M. HORVATH. 375 pp., $6.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y., 1960.

Group Processes. Transactions of the Fifth Conference October 1958. Edited by BERTRAM SCHAFFNER. 196 pp., $4.50. The Josiah Macy, Jr. Foundation Publications, New York, N. Y., 1960.

The Heart in Industry. Edited by LEON J. WARSAW. Foreword by Irving S. Wright. 677 pp., $16.00. Paul B. Hoeber, Inc., Medical Division of Harper & Bros., New York, N. Y., 1960.

Report of the Medical Research Council for the Year 1958–1959. Presented to Parliament by the Minister of Science by Command of Her Majesty, June, 1960. 330 pp., 15s 6d net. Her Majesty's Stationery Office, London, England.

Surgical Errors and Safeguards, 5th edition. By MAX THOREK. 652 pp., $25.00. J. B. Lippincott Co., Philadelphia, Pa., 1960.

The Tonsils and Adenoids in Childhood. By DONALD F. PROCTOR. Edited by Mark M. Ravitch. 70 pp., $7.50. Charles C Thomas, Publisher, Springfield, Ill., 1960.

PANCREATITIS IN CHILDREN FOLLOWING ADRENAL CORTICO-STEROID[1] THERAPY[2]

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The discovery in 1955 that pancreatitis occurs in rabbits following the administration of cortisone (1, 2) alerted pathologists to the possibility of finding similar lesions in man. Noteworthy is the report of Carone and Liebow (3), who reviewed the autopsy material from fifty-four adults who had received adrenal cortico-steroids, and found focal pancreatic lesions in over 50 per cent. Sixteen of these patients had acute pancreatitis with fat necrosis. Recently pancreatitis occurring while on steroids has been recognized during life (4); and a report of six clinical cases from this hospital is now in preparation (5).

Despite the apparent frequency of this complication of cortisone therapy in adults, it has been described only three times in children. Baar and Wolff (6) reported an eleven year old girl who had been on cortisone for asthma and showed acute pancreatitis at autopsy, and a three year old boy given steroids for dermatomyositis who had pancreatitis at necropsy. Marczynska-Robowska (7) reported the same lesion in a five year old boy with Still's disease and terminal smallpox, who had been receiving cortisone.

Focal pancreatic lesions are known to occur in children with lipoid nephrosis as well as in those dying in uremia resulting from other renal abnormalities (8). These lesions are incidental and of secondary importance. In the years after introduction of adrenal steroid therapy, the incidence and severity of pancreatic lesions in this hospital have seemed to increase and even to assume a primary role as the cause of death in some children. Prolonged therapy with these agents has been given mainly to children with nephrosis or leukemia. The pancreatic findings in each child dying with one of these diseases have been reviewed and the cases autopsied before and after the therapeutic use of cortisone have been compared.

STUDY MATERIAL

Group I consists of children with chronic lipoid nephrosis who died during the years 1945–54. The vast majority of the cases of "pure" nephrosis, autopsied before that time

[1] No distinction has been made between the various commercial preparations used in this study.

[2] Photomicrographs for this article are by Mr. Robert Laughlin.

(15 of 17 cases) died as a result of pneumococcal peritonitis before renal insufficiency developed. Associated with the peritonitis there was acute bacterial inflammation in the interstitial tissue of the pancreas making it necessary to omit these earlier cases from this study. Since 1945 sulfonamides and antibiotics prolonged the lives of the patients and instead of dying of infection, they developed varying degrees of azotemia. As only twelve children comprise this group, too small a number to be of statistical value, fifteen children dying in sequence (1935–1955) in uremia from miscellaneous causes have been added to it. None had received cortisone. These twenty-seven children served as controls.

Group II consists of twenty-five children with the nephrotic syndrome that had been given steroids in addition to antibiotics.

In Group III, there are twenty-five children, selected in sequence, dying with leukemia, a tumor or aplastic anemia prior to the advent of steroid therapy. They are contrasted to the twenty-five children in Group IV who had similar diseases, but had been receiving adrenal corticosteroid therapy.

RESULTS

In Group I, twenty-seven children died in uremia without having received steroid therapy (Table I). Twelve children died with intercapillary ("membranous") glomerulonephritis following a nephrotic syndrome of six months to six years duration. One of these children had a small pancreatic duct occluded by squamous metaplasia, and nearby was a focus of chronic interstitial pancreatitis. (Another child, ill four years, showed thickening of the peritoneal surface adjacent to the pancreas without any lesion in the pancreas. This was interpreted as the result of bacterial peritonitis early in the course of the disease). In the fifteen children dying in uremia from various causes (five subacute glomerulonephritis; four chronic glomerulonephritis; four hydronephrosis and chronic pyelonephritis with congenital malformations of the lower urinary tract; and two congenital cystic kidneys), three showed focal pancreatic lesions, that is, inspissated secretions, dilated ductules and acini, and chronic interstitial pancreatitis (Fig. 1). No instance of acute pancreatitis or fat necrosis was found. The incidence of focal chronic pancreatic lesions for this entire control group (four in twenty-seven cases) was 14.8 per cent.

Table II summarizes the findings in twenty-five nephrotic children who had received adrenal cortico-steroids for a period of two weeks to several years. In these children, the kidney lesion had progressed from "pure" nephrosis to intercapillary glomerulonephritis; and all showed varying degres of renal failure before death. Three of these children had diffuse hemorrhagic pancreatitis with wide-spread fat necrosis at autopsy (Fig. 2); seven other children showed focal pancreatic lesions consisting of organizing fat necroses, focal pancreatic scarring, atrophy and necrosis, inspissated secretions, and dilatation of ductules and acini (Fig. 3). The incidence of pancreatic lesions in the cortisone-treated children was 40 per cent (ten in twenty-five cases) or more than two and one-half times that for the chil-

TABLE I

Children dying with "lipoid nephrosis" or with uremia secondary to other renal disease. (1935–1955). No history of steroid or ACTH therapy.

JHH Autopsy Number	Age	Duration of Symptoms	Renal Disease	Pancreatic Lesions
22374	3 yrs.	1 yr.	"Nephrotic nephritis"	Squamous metaplasia of one duct. Focal chronic pancreatitis
18837	11 yrs.	Several years	Chronic glomerulonephritis	Focal dilatation of ductules and acini
25293	2 yrs.	2 yrs.	Congenital cystic kidneys	Focal inspissation of secretions with dilatation of ductules and acini
21380	5 yrs.	5 yrs.	Hydronephrosis and chronic pyelonephritis	Focal chronic interstitial pancreatitis
21959	2 yrs.	1 yr.	"Nephrotic nephritis"	None
22548	2 yrs.	1½ yrs.	"Nephrotic nephritis"	None
20984	3 yrs.	1½ yrs.	"Nephrotic nephritis"	None
19410	4 yrs.	71 yrs.	"Nephrotic nephritis"	None
20079	4 yrs.	2 yrs.	"Nephrotic nephritis"	None
22406	4 yrs.	2½ yrs.	"Nephrotic nephritis"	None
19911	5 yrs.	6 mos.	"Nephrotic nephritis"	None
22509	5 yrs.	2 yrs.	"Nephrotic nephritis"	None
19942	6 yrs.	2 yrs.	"Nephrotic nephritis"	None
22883*	8 yrs.	4 yrs.	"Nephrotic nephritis"	None (Peritoneal scarring**)
25492	11 yrs.	6 yrs.	"Nephrotic nephritis"	None
14388	4 yrs.	>7 mos.	Chronic glomerulonephritis	None
14644	6 yrs.	>1 yr.	Chronic glomerulonephritis	None
24097	10 yrs.	?	Chronic glomerulonephritis	None
23737	6 mos.	2½ mos.	Subacute glomerulonephritis	None
20815	16 mos.	2 mos.	Subacute glomerulonephritis	None
16369	2 yrs.	? 2 wks.	Subacute glomerulonephritis	None
21846	6 yrs.	6 mos.	Subacute glomerulonephritis	None
18572	8 yrs.	1 month	Subacute glomerulonephritis	None
23185	2 mos.	2 mos.	Congenital cystic kidneys	None
24224	1 month	1 month	Hydronephrosis	None
23339	4 yrs.	4 yrs.	Hydronephrosis	None
26135	5 yrs.	5 yrs.	Chronic pyelonephritis	None

* Received 100 mg. of cortisone on day of death.

** Probably due to remote peritonitis.

dren with renal disease who had not received steroids. The presence or severity of the lesion did not appear to be related to the duration of steroid therapy or the doses administered.

Group III consists of twenty-five children; twenty with leukemia, three with aplastic anemia and one with lymphosarcoma, dying before the advent of cortisone therapy. None of these children showed any independent pancreatic lesion, except the presence of tumor invasion (Table III). On the other hand, in twenty-five children with similar ailments (sixteen with

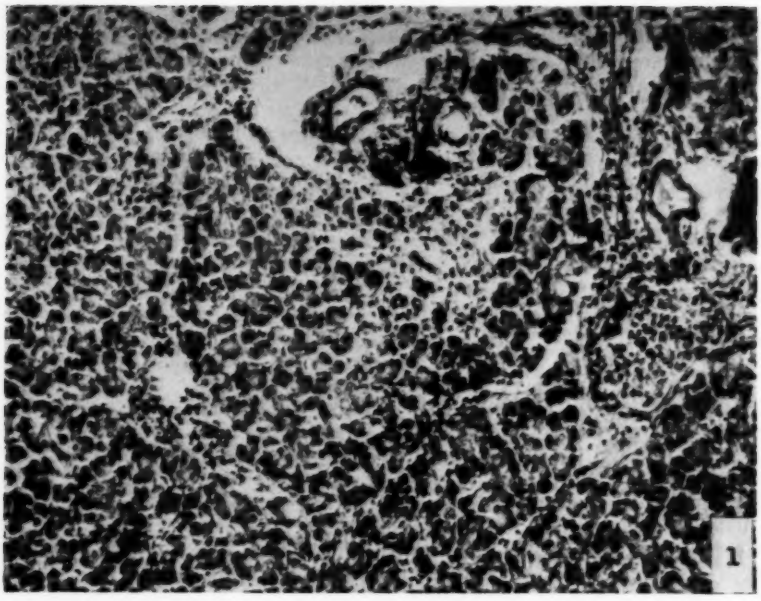

FIG. 1. Autopsy 22374: Three year old child with lipoid nephrosis for 1 year. Section of pancreas ×160, showing focus of dilated ductules and chronic interstitial pancreatitis.

leukemia, four with tumors and five with aplastic anemia) receiving ACTH or cortisone, for periods of three weeks to eight months, four or 16 per cent showed focal pancreatic lesions (Fig. 4) consisting of inspissated secretions and focal dilatation of ductules and acini in sections of pancreas not involved by tumor (Table IV).

DISCUSSION

Although this is a small series, it is apparent that there has been an increase in pancreatic lesions in children with renal disease or blood dyscrasias since the introduction of cortisone therapy. Before the use of this treatment, no pancreatic lesions were seen in twenty-five children with leukemia, tumors or aplastic anemia; in association with this or similar therapy 16 per cent of twenty-five similarly ill children showed focal pancreatic lesions at autopsy. A review of the clinical charts of these children failed to uncover any predisposing factor except the adrenal cortico-steroid therapy. Hypercholesterolemia was not observed.

The incidence of pancreatic lesions in the steroid-treated children with renal disease is much higher than in the leukemic children given steroids. Forty per cent of this group (ten of twenty-five children) had pancreatic in-

TABLE II

Children dying with "nephrotic nephritis". History of steroid therapy.

JHH Autopsy Number	Age	Duration of Symptoms	Duration of Steroid Therapy	Cause of Death*	Pancreatic Lesions
23529	2 yrs.	6 mos.	1 month	Acute pancreatitis	Old and fresh pancreatitis with fat necroses
25354	3 yrs.	1 yr.	1 yr.	Acute pancreatitis and generalized acute cryptococcosis	Old and fresh pancreatitis with fat necroses
28531	11 yrs.	10 mos.	10 mos.	Acute pancreatitis with abdominal hemorrhage	Old and fresh pancreatitis with fat necroses
23737	6 mos.	6 wks.	1 month	Cerebral thrombi and pulmonary emboli	Focal inspissation of secretions, acinar atrophy and necroses
28867	13 mos.	12 mos.	9 mos.	Uremia	Organizing fat necrosis
29266	2 yrs.	6 mos.	6 mos.	Uremia	Organizing fat necrosis
26396	4 yrs.	2½ yrs.	2½ yrs.	Uremia	Diffuse inspissation of secretions with dilatation of ductules and acini
28105	4 yrs.	2 yrs.	2 yrs.	Uremia	Focal dilatation of ductules and acini
25005	7 yrs.	4½ yrs.	"Several years"	Uremia	Focal scarring and atrophy
24136	14 yrs.	4 mos.	4 mos.	Hemorrhage from duodenal ulcer	Chronic pancreatitis and organizing peripancreatic fat necrosis
27305	4 mos.	3 wks.	2 wks.	Uremia. Died during febrile reaction following blood transfusion	None
28528	6 mos.	6 mos.	5 mos.	Septicemia and meningitis	None
26567	21 mos.	6 wks.	6 wks.	Renal vein thrombosis with uremia	None
23055	2 yrs.	7 mos.	3 mos.	Sudden, unexplained death during paracenteses	None
25575	2 yrs.	5 mos.	5 mos.	Pneumonia and chronic glomerulonephritis	None
24483	3 yrs.	18 mos.	1 year	Uremia	None
25936	3 yrs.	16 mos.	4 mos.	Pneumonia and chronic glomerulonephritis	None
23265	5 yrs.	22 mos.	10 mos.	Uremia	None
23302	5 yrs.	2 yrs.	15 mos.	Uremia	None
26227	6 yrs.	3½ yrs.	2 yrs.	Uremia and pneumonia	None
24048	7 yrs.	6 mos.	5 mos.	Uremia	None
26949	8 yrs.	17 mos.	5 mos.	Renal vein thrombosis with septic pulmonary emboli. Uremia	None
28272	11 yrs.	9 yrs.	"Many years"	Uremia	None
28195	12 yrs.	2 yrs.	3 mos.	Uremia	None
28224	12 yrs.	4 mos.	4 mos.	Uremia	None

* Glomerular lesions were present in every case, varying from focal acute lesions to typical chronic glomerulonephritis.

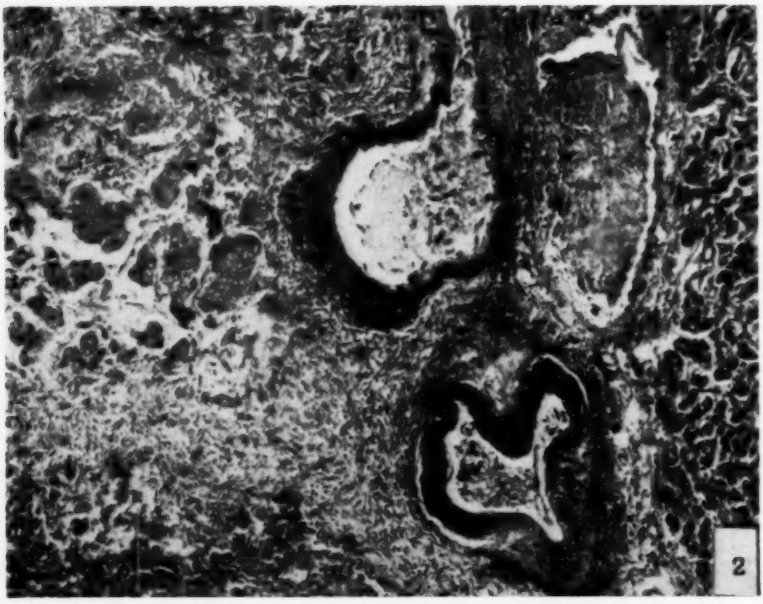

FIG. 2. Autopsy 28531: Eleven year old child; lipoid nephrosis with steroid therapy for 10 months. Had repeated episodes of abdominal pain. Section of pancreas ×100, showing pancreatic necrosis, with partial necrosis of intrapancreatic vessels.

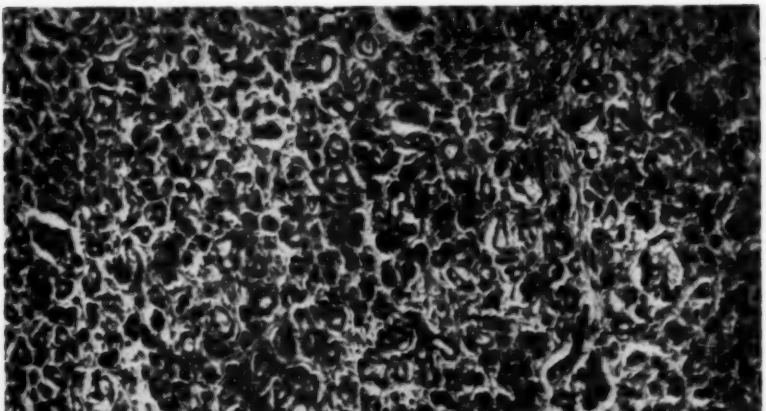

FIG. 3. Autopsy 26396: Four year old nephrotic; symptoms and steroids for 2½ years. Section of pancreas ×100, showing ductular and acinar dilatation; slight interstitial pancreatitis.

TABLE III

Children dying with leukemia, aplastic anemia or tumor. No history of steroid or ACTH therapy.

JHH Autopsy Number	Age	Duration of Symptoms	Disease	Pancreatic Lesions Unassociated With Tumor
21240	7 mos.	2 mos.	Acute myeloid leukemia	None
19457	11 mos.	?	Acute myeloid leukemia	None
20567	2 yrs.	>2 mos.	Acute myeliod leukemia	None
21824	2 yrs.	3 mos.	Acute myeloid leukemia	None
21782	8 yrs.	1 month	Acute myeloid leukemia	None
21164	10 yrs.	2 mos.	Acute myeloid leukemia	None
19630	14 yrs.	2 mos.	Acute myeloid leukemia	None
22054	8 mos.	2½ mos.	Acute lymphoid leukemia	None
20516	18 mos.	2 mos.	Acute lymphoid leukemia	None
22397	21 mos.	2½ mos.	Acute lymphoid leukemia	None
22296	2 yrs.	>2 mos.	Acute lymphoid leukemia	None
19014	3 yrs.	2 mos.	Acute lymphoid leukemia	None
22300	4 yrs.	2 mos.	Acute lymphoid leukemia	None
19574	5 yrs.	5 mos.	Acute lymphoid leukemia	None
20417	6 yrs.	3 mos.	Acute lymphoid leukemia	None
20954	6 yrs.	>3 mos.	Acute lymphoid leukemia	None
22286	8 yrs.	9 mos.	Acute leukemia	None
22149	4 yrs.	11 mos.	Chronic lymphoid leukemia	None
19264	5 yrs.	2 mos.	Chronic lymphoid leukemia	None
22002	7 yrs.	1 year	Chronic lymphoid leukemia	None
21398	11 yrs.	2½ mos.	Chronic lymphoid leukemia	None
21859	3 yrs.	1 year	Aplastic anemia	None
20906	4 yrs.	>3 mos.	Aplastic anemia	None
20709	10 yrs.	4 mos.	Aplastic anemia	None
20866	3 yrs.	6 mos.	Lymphosarcoma	None

volvement, and three of these children showed severe acute hemorrhagic pancreatitis. Prior to the introduction of steroid therapy, four of twenty-seven children with renal disease or 14.8 per cent showed incidental focal chronic pancreatic lesions at autopsy. It is possible that this may be related to dehydration or electrolyte imbalance. However a survey of the clinical charts of these children revealed no specific abnormal pattern of serum electrolytes. Repeated paracenteses, a routine procedure for the ascites associated with nephrosis, is also a possible contributing factor. Another cardinal symptom of nephrosis is hyperlipemia (8) and it is possible that this chemical alteration predisposes to inspissation of pancreatic secretions. It has been noted previously that pancreatitis occurs with essential hyperlipemia (9). Furthermore, cortisone administration has been known to cause an elevation of the blood lipids both in man (10) and in experimental animals (11). However in the present series of cases, although the serum cholesterols are high, there is no correlation between the cholesterol level and the presence of pancreatic lesions.

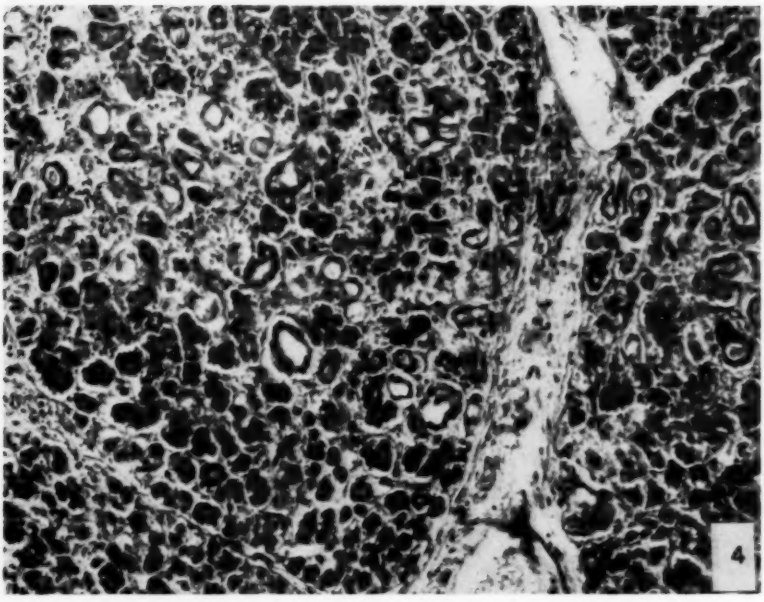

FIG. 4. Autopsy 23359: Twelve year old child with aplastic anemia of 2 months duration; steroid therapy for 3 weeks. Section of pancreas ×100, showing focal marked dilatation of ductules and acini, and slight interstitial pancreatitis.

Whatever their cause, focal pancreatic lesions in children with renal disease receiving no cortisone may explain the greater susceptibility to more serious pancreatic disease following steroid therapy. In the three cases dying with acute hemorrhagic pancreatitis, there had been previous episodes of abdominal pain, and at autopsy in addition to the acute pancreatitis, there were foci of healed pancreatitis and organized fat necroses. It is often presumed that abdominal pain with cortisone therapy indicates the presence of peptic ulcer, but as can be seen in Table II, peptic ulcer occurred only once in this series, and undoubtedly the diagnosis of pancreatitis should always be considered when this symptom occurs.

The mechanism of cortisone injury to the pancreas has not been explained by experimental studies. It has been suggested that obstructing epithelial proliferation in pancreatic ductules may be the cause of the cortisone-produced pancreatitis in rabbits (2), but inspissated secretions were also noted by these investigators. In the steroid-treated nephrotic children, most of the focal pancreatic lesions are associated with inspissated secretions, with resulting dilatation of ductules and acini. Further lesions result from rupture of ductules, dissemination of pancreatic enzymes with

TABLE IV

Children dying with leukemia, aplastic anemia or tumor. History of steroid or ACTH therapy.

JHH Autopsy Number	Age	Duration of Symptoms	Duration of Steroid or ACTH Therapy	Disease	Pancreatic Lesions Unassociated with Tumor
28352	2 yrs.	8 mos.	8 mos.	Acute leukemia	Focal dilatation of ductules and acini
26298	10 yrs.	20 mos.	Several months	Acute leukemia	Focal dilatation of ductules
26791	9 yrs.	6 mos.	6 mos.	Aplastic anemia	Focal dilatation of ductules and acini
23359	12 yrs.	2 mos.	3 wks.	Aplastic anemia	Diffuse inspissation of secretions with dilatation of ductules and acini
23115	3 yrs.	6 mos.	Several months (ACTH)	Acute lymphoid leukemia	None
24188	4 yrs.	7 mos.	7 wks. (ACTH)	Acute lymphoid leukemia	None
27908	4 yrs.	9 wks.	8 wks.	Acute lymphoid leukemia	None
22491	5 yrs.	15 mos.	4 mos. (ACTH)	Acute lymphoid leukemia	None
22574	14 mos.	4 mos.	3 wks. (ACTH)	Acute leukemia	None
27835	4 yrs.	5 mos.	5 mos.	Acute leukemia	None
26280	7 yrs.	18 mos.	2 mos.	Acute leukemia	None
27274	8 yrs.	3 mos.	3 mos.	Acute leukemia	None
28695	11 yrs.	8 mos.	8 mos.	Acute leukemia	None
29243	13 yrs.	5 mos.	5 mos.	Acute leukemia	None
27407	7 yrs.	1 year	1 month	Monocytic leukemia	None
25939	2 yrs.	6 mos.	4 mos.	Chronic myeloid leukemia	None
23646	15 yrs.	2 yrs.	Several months	Chronic myeloid leukemia	None
24010	3 yrs.	18 mos.	Several months (ACTH and cortisone)	Aplastic anemia	None
22526	7 yrs.	3 mos.	1½ mos.	Aplastic anemia	None
23355	8 yrs.	6 yrs.	18 mos. (ACTH)	Aplastic anemia	None
27582	14 yrs.	14 yrs.	Several months	Aplastic anemia	None
26766	12 yrs.	1 year	1 month	Lymphosarcoma	None
28308	4 yrs.	4 mos.	?	Neuroblastoma	None
27884	5 yrs.	>5 mos.	2 mos.	Sarcoma	None
23274	2 yrs.	5 mos.	3 mos.	Hand-Schuller Christian's disease	None

fat necroses, inflammation, and in the most serious cases, necrosis of vessel walls and hemorrhage. Only the milder focal pancreatic lesions are noted in the steroid-treated leukemic and anemic children. No proliferation of

duct epithelium was seen in either group of children following cortisone therapy. It is interesting, however, that duct hyperplasia occurred in one child with lipoid nephrosis prior to the introduction of cortisone therapy.

SUMMARY

In children with renal disease, the autopsy incidence of pancreatic lesions increased from 14.8 per cent in twenty-seven children without adrenal cortico-steroid therapy to 40 per cent in twenty-five children following this form of therapy. Three of the latter children showed acute hemorrhagic pancreatitis at autopsy.

In twenty-five children with leukemia, tumors or aplastic anemia prior to cortisone therapy no pancreatic lesions were seen except those due to direct tumor invasion; in twenty-five children with similar diseases treated with ACTH or steroids, the incidence of incidental focal pancreatic lesions was 16 per cent.

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FURTHER OBSERVATIONS ON THE LOCALIZATION OF GLYCOGEN IN FROG STRIATED MUSCLE[1]

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Recently Bergman and Walker (1) reported on the cytochemical localization of oxidative enzymatic activity and glycogen in frog skeletal muscle. On the basis of the distribution of the diastase-labile PAS-positive material it was suggested that glycogen was deposited in the interfibrillar spaces and in the myofibrillar A band and Z line. In an early study, Arnold (2) reported that glycogen was found in the interfibrillar spaces adjacent to the Z lines and I bands. No myofibrillar glycogen was reported. Dempsey, Wislocki and Singer (3) and Mancini (4), on the other hand, reported glycogen to be localized within the I band on both sides of a glycogen negative Z line. Goldstein (5) has reviewed the literature in some detail concerning the status of PAS staining in striated muscles and reported on an extensive study of skeletal and cardiac muscle. The results of this investigation suggested that glycogen appeared to lie against the Z line, or more often was distributed throughout the sarcomere or I band alone. Goldstein also observed that a "saliva resistant" PAS-positive material which he believed to be an acid mucopolysaccharide was found in the Z line, intercalated discs, cell borders, A bands and elsewhere. The literature review by Goldstein reveals that little agreement has been reached regarding the distribution of glycogen in striated muscles.

Fawcett and Selby (6) have provided evidence that localization of glycogen stained with the PAS method, as seen with the light microscope, corresponds to the localization of lead hydroxide staining, as observed with the electron microscope. The possibility of the use of lead hydroxide as a stain for glycogen has also been discussed by Watson (7).

In the present study, the localization of PAS and lead hydroxide positive substances in striated muscle has been examined with the aid of light and electron microscopy.

MATERIALS AND METHODS

The sartorius muscles from frogs (Rana pipiens) were fixed for light microscopy in the following ways: 1). In isotonic, buffered (pH 7.2), 1 per cent osmium tetroxide at

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FIG. 1. Electron micrograph of frog sartorius muscle fixed in buffered (pH 7.2) isotonic, 1 per cent osmium tetroxide without lead hydroxide staining. Glycogen granules can be seen in the interfibrillar spaces in close association outside the sarcoplasmic reticulum. They are not selectively stained. S, sarcomere; CU, basic contractile unit in this muscle; A, A band; H, H zone; M, M line; I, I band; Z, Z line; Mi, mitochondrion and SR, sarcoplasmic reticulum. ×45,000.

0°C., 2). Osmium vapor, 3). Absolute alcohol at 0°C., 4). Bouin's fluid at 0°C., and 5). Rossman's fluid at 0°C. The time of fixation with osmium varied from ½ to 4 hours while the time for non-osmium fixation was 24 to 30 hours.

Fixation of tissue at room temperature (27°C.) was not found to be satisfactory because of the lack of uniformity of PAS staining due probably to diffusion shifts of interfibrillar glycogen.

Samples of the tissue fixed by these agents were also studied by electron microscopy. Because non-osmicated tissues usually do not withstand methacrylate embedding, some of the tissue fixed in Rossman's fluid, absolute alcohol, and Bouin's fluid were run down and rinsed in distilled water and post-osmicated for 30 minutes in osmium vapor or in buffered (pH 7.2), 1 per cent osmium tetroxide. Following the fixation period, the tissues were dehydrated either in graded alcohols or placed directly in absolute alcohol. After thorough dehydration the tissues were infiltrated with a mixture (1:9) of methyl and butyl methacrylate. Methacrylate polymerization was carried out in an oven at 45 to 55°C. Tissue samples were also embedded in paraffin.

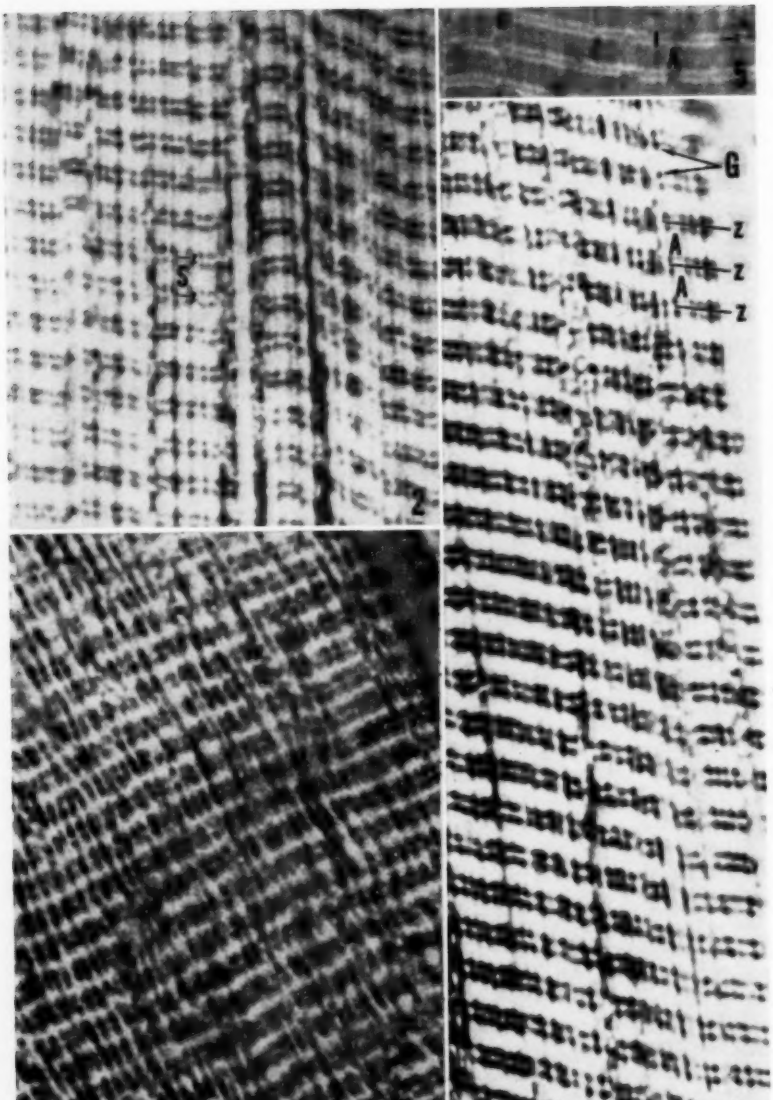
Muscle embedded in plastic was sectioned by means of glass knives and a Porter-Blum microtome. Sections for light microscopy were approximately one-half micron or less in thickness and were stained or otherwise prepared without the removal of the methacrylate embedding medium. The paraffin embedded tissues were cut approximately one micron thick.

The PAS method employed for the demonstration of glycogen with the light microscope was that recommended by Lillie (8). Prior to PAS staining, control sections were incubated for 1 to 4 hours at 37°C. in a 0.1 to 0.3 per cent solution of purified malt diastase (U.S.P) buffered with Sorenson's phosphates (0.02M) at pH 6.0. In addition, control sections were also incubated in saliva for 1 to 4 hours at 37°C.

The tissue sections were stained with lead hydroxide according to the method of Watson (7) or examined without lead hydroxide treatment. In addition, some sections (to serve as experimental controls) were exposed to diastase (as cited above) before lead hydroxide treatment. The preparations were studied and photographed by means of an RCA EMU 3 c electron microscope.

Electron micrographs taken from diastase-treated thin sections of skeletal muscle reveal the absence of lead hydroxide stained granules. It should be noted that diastase digestion of thin, methacrylate embedded, muscle sections requires long periods, approximately 3 hours at 37°C. After this period most of the muscle cells, but not the connective tissue, are PAS-negative. It takes from 1 to 2 hours longer (approximately 4 to 5 hours) for the sections to become PAS-negative when exposed to distilled water (9) or to the buffer solution without diastase. In ordinary paraffin embedded tissue sections, commonly used for light microscopy (1 to 5 microns thick) the digestion time (after removal of paraffin) may be as little as 30 minutes or as much as 3 hours. These thick tissue sections exposed to buffer alone (no enzyme) require much longer periods to obtain complete PAS-negativity. The connective tissue, however, remains PAS-positive. Plastic embedded tissues apparently require longer periods to complete the digestion of glycogen. Salivary digestion at 37°C. yields results similar to those obtained with diastase, although the results are so spotty that some areas appear completely digested while other areas still remain PAS-positive. Saliva is a poor source of amylase for an experimental control, because its concentration is not known, it may contain other enzymes [such as ribonuclease (6)], is found in a viscid, mucous-containing medium, and does not yield optimal results. It is interesting to note that the digestion of glycogen granules is extremely difficult in tissues fixed first by osmium vapor.

The intracellular PAS-positive material of the striated skeletal muscle cell has a char-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acteristic distribution between and within the myofibrils. The PAS-positive material appears to have the same localization as that stained by lead hydroxide and observed in the electron microscope.

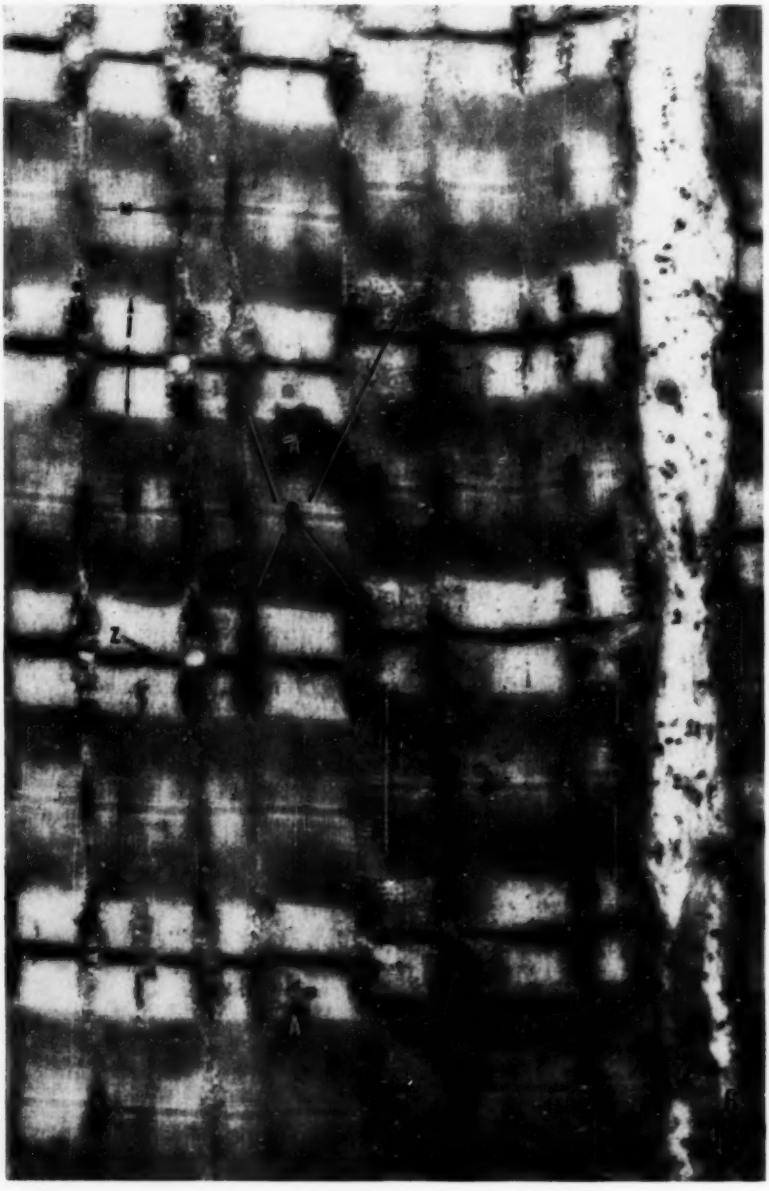
RESULTS

Glycogen appears to be localized within the interfibrillar spaces and within the myofibrils. The distribution pattern of each will be described separately.

1. The interfibrillar distribution of glycogen.

The most striking distribution of glycogen is within the interfibrillar spaces because its high concentration there yields intense staining by the PAS method. The interfibrillar space varies because of the periodic changes in dimension of the myofibrils. In well-fixed tissue the interfibrillar space is very much reduced in the region of the A band and enlarged in the region of the I band. In ½ micron sections the PAS staining appears as tear-shaped dots adjacent to the Z line and I band. The tails point toward the M line of the A band. This corresponds exactly to the interfibrillar spaces. See Figures 2, 3, and 4. In thick sections (1μ) the dots appear to be continuous laterally and obscure the I band. See Figure 4. Because of the variations in dimension in the interfibrillar spaces the myofibrils appear to be surrounded by a PAS-positive cuff in the region of the I band. The Z line which is continuous across the cell, subdivides the interfibrillar space and defines the sarcomere. Thus the sarcomere will appear to be bounded (in half micron sections) by 4 PAS-positive dots. This pattern is repeated throughout the muscle fiber. See Figures 2, 3, and 4. Electron micrographs of lead hydroxide-stained tissue confirms the described distribution of the interfibrillar glycogen (Fig. 6). The size (approximately 150 to 500 A. in diameter) of these interfibrillar lead hydroxide positive "granules" corresponds to those described by Fawcett and Selby (6). The pattern described above can be most readily obtained with any non-aqueous fixative. Osmium vapor appears to be the best fixative for this purpose, although after osmium

FIGS. 2, 3, 4 and 5. Figures 2 and 4 are osmium-fixed, PAS-stained, ½ micron sections of frog sartorius muscle. The basic pattern is a transverse double row of PAS-positive material separated by a thin unstained line. The glycogen (G) is located in the interfibrillar spaces adjacent to the I band (I). The non-stained thin zone is occupied by the telophragma or the Z line (Z). The broad, unstained area is the A band (A). Note that the stained areas are sometimes connected in the longitudinal plane by a thin streak. In addition, the stained areas may also appear joined in the transverse plane. Thus the sarcomere (S) is outlined by the PAS-staining. Figure 3 shows a pattern similar to those described above but the muscle was fixed in picric-alcohol, post-osmicated and PAS-stained. Figure 5 is PAS-stained, Bouin's-fixed, post-osmicated muscle and shown for comparison with Figures 2, 3, and 4. ×2,750.



treatment post-fixation digestion of glycogen by saliva or amylase is very difficult.

2. The intrafibrillar distribution of glycogen.

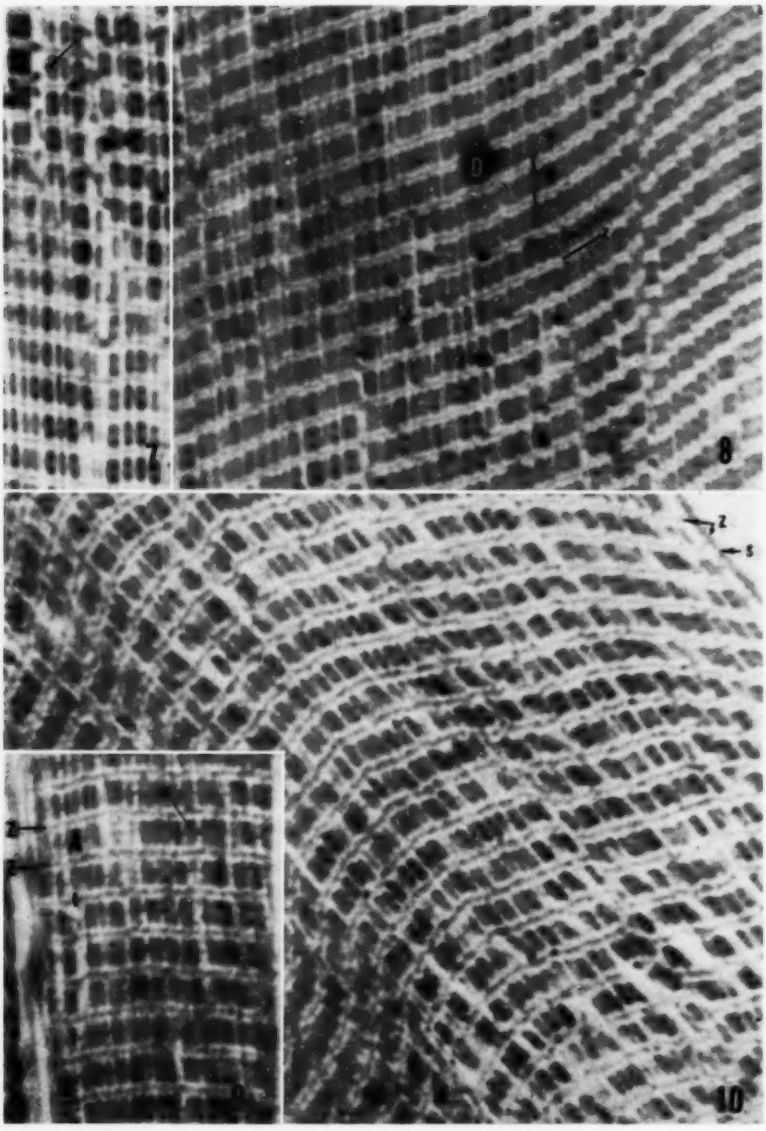
The PAS-positive, diastase labile material within the A band and Z line was found to be very much less concentrated than that within the interfibrillar spaces. It is usually so faint (PAS stain) that it is not easily recognized unless the interfibrillar glycogen is removed. This can usually be accomplished by the use of aqueous fixatives and washing in water after fixation of the tissue. Bouin's fixation most readily reveals the intramyofibrillar PAS-positive material. It is not unusual to find a muscle showing some interfibrillar staining in addition to the myofibrillar pattern. The differences between the two distribution patterns are easily recognized in thin sections (½ micron).

In electron micrographs of tissue sections prepared by osmium vapor or isotonic, buffered (pH 7.2) 1 per cent osmium tetroxide with subsequent lead hydroxide counterstaining, several observations can be made which appear to correlate with the light micrographs. The lead hydroxide-stained interfibrillar glycogen (spheres approximately 150 to 500 A. in diameter) is actually aggregation of small granules 75 to 100 A. in diameter (see electron micrographs, Fig. 11). The A band and Z line contain numerous small (non-aggregated) granules which appear to be embedded in the sarcoplasm of these areas. It should be noted that small dense glycogen granules are found within the I band but are not nearly as numerous (Fig. 11). If it is true that lead hydroxide stains the intramyofibrillar glycogen somewhat selectively then it is probable that the I band merely appears PAS-negative because of its very low concentration of glycogen.

Both the large (150 to 500 A.) aggregates and the smaller (75 to 100 A.) dispersed granules are saliva and diastase labile (Fig. 12).

Additional observations reveal that there may be irregular or spotty (but never non-specific) PAS stainings within the muscle cell (prior to enzymatic digestion) which are probably due to extraction artifacts resulting from the fixation of the muscle. Such irregularity is sometimes seen in tissues fixed in Bouin's fluid, absolute alcohol and picric-alcohol. These "artifacts" apparently do not occur in osmium-fixed muscle tissues. Spotty or irregular staining of the interfibrillar spaces probably results from the

FIG. 6. An electron micrograph of frog sartorius muscle fixed in osmium vapor and treated with lead hydroxide. The lead hydroxide "granules" are found primarily in the area adjacent to the I band and Z line. Although most glycogen "granules" appear to be outside the endoplasmic reticulum some occasionally do appear within the tubular system of the reticulum. Compare with Figures 1, 3 and 4. In the fifth myofibril from the left the granules are disposed in the space over the I band. White A, artifact; black A, A band; I, I band; H, H zone; Z, Z line; M, M line and G, glycogen. ×23,000.



FIGS. 7, 8, 9, and 10. Light micrographs of PAS-stained frog sartorius muscles fixed in absolute alcohol, hydrated for post-fixation in osmium vapor (Fig. 7). Arrow indicates interfibrillar glycogen. Figures 8, 9 and 10 are Bouin's-fixed, rinsed in water, post-osmicated, PAS-stained sartorius muscle. The PAS staining is clearly myofibrillar and reveals the A band (A), transversely continuous Z line or telophragma (Z), and M line or mesophragma (M) and the sarcolemma (S). The H zone (H) is not as strongly stained as the remainder A band (Fig. 8). The I band (with the exception of the Z line or telophragma) appears PAS-negative. The mesophragma (M) is demonstrated in Figure 9. Dust (D) artifacts (not PAS-staining) are seen in Figure 10. ×2,750.

FIG. 11. High magnification electron micrograph of osmium vapor-fixed, lead hydroxide-treated sartorius muscle. Large glycogen "granules" (G) are seen in the upper left side of the micrograph. The large "granules" vary in size from 150 to 500 A. and appear as aggregates of smaller granules 75 to 100 A. in diameter. The small glycogen granules are dispersed throughout the A band and Z line. In addition, some of the small granules can be found in the I band. A, A band; M, M line; I, I band; Z, Z line and G, glycogen granules. ×72,000.

FIG. 12. Electron micrograph of osmium-fixed, diastase-treated, lead hydroxide-stained ultra thin sections. Note the absence of glycogen granules from the intermyofibrillar spaces and myofibrils. The appearance of numerous artifacts results from the diastase and lead hydroxide treatment. The thoroughly successful hydrolysis of the glycogen must be related to the extremely thin sections used for electron microscopy since thicker plastic sections of osmium-fixed tissue are not easily digested by diastase. ×24,000.

destruction or incomplete extraction of the contents of the interfibrillar spaces during fixation or during hydration of the tissue during post-osmication, as described earlier in this section. The artifacts described do not in any way result in non-specific staining in terms of the two basic patterns of glycogen distribution described above. In no instance was spotty or irregular staining of the intercellular connective tissue encountered regardless of the fixation method employed.

DISCUSSION

The results of this study suggest that glycogen is localized principally in the interfibrillar spaces and also in the myofibrillar Z line and A band. These observations are made possible through the study of very thin sections (approximately 0.5 micron thick or less) for light microscopy and ultrathin sections for electron microscopy. The findings observed with the PAS reaction have been correlated with electron microscope observations using the lead hydroxide technique of Watson (7). It is clear that the lead hydroxide method has numerous pitfalls because it is not specific for glycogen and is therefore subject to the production of numerous artifacts and misinterpretation. Because it is possible to correlate the observations made by the PAS and lead hydroxide methods through the use of light and electron microscopy, and since the patterns described with the two methods are diastase-labile it is felt that the lead staining has made possible a more precise localization of glycogen than was possible before.

In the numerous reports describing the distribution of glycogen (see Goldstein's paper) in striated skeletal muscle, one of the chief areas of controversy concerns the possible high glycogen content of the I band. The present study reveals that a high concentration of glycogen is contained in the interfibrillar spaces in close association with the sarcoplasmic reticulum which encircles the myofibrillae at the I band. It is not possible that such a distribution can be discerned by use of sections thicker than 1 to 2 μ. This study reveals that the interfibrillar glycogen is preserved best by osmium tetroxide and by picric-alcohol fixation. The results yielded by these fixatives apparently mask to a great extent the weaker myofibrillar staining. Removal of the contents of the interfibrillar spaces from tissues fixed in Bouin's fluid or picric-alcohol as a result of washing in distilled water and during post-osmication permits the visualization of the Z lines and A bands by the PAS method. Because the staining pattern is very uniform, specific and consistent, it is suggested that these observations contribute to our knowledge of glycogen distribution in living muscle.

It is interesting to note that early electron microscope studies of striated skeletal muscles (10, 11, 12, 13) described the sarcoplasmic reticulum as

associated with granules not unlike the RNA granules attached to the endoplasmic reticulum of other cells (e.g., pancreatic acinar cells). Fawcett and Selby (6) suggested that these granules may actually be glycogen on the basis of their study of glycogen in the turtle heart. The present report supports this interpretation.

It has been observed (3 and 5) that the PAS-positive material located in the "I band and piled-up against the Z line" is more readily soluble or digestible than the so-called "diastase-resistant" material that remains within the other cross bands. A plausible explanation for this interpretation appears to be offered by the appearance of the glycogen as revealed by electron microscopy. The large glycogen aggregates (150 to 500 A. in diameter) are not "trapped" by the fixation of the muscle protein since they actually occupy the interfibrillar "spaces." On the contrary, the dispersed phase of glycogen granules appear to be embedded in the sarcoplasm associated with the myofibrils, primarily in the A band and Z line. As a result, the enzyme diastase or salivary amlyase probably does not have easy access to this glycogen. The suggestion (5) that the A band and Z line contains an acid mucopolysaccharide does not appear justified.

The observations suggest that the interfibrillar glycogen is composed of aggregated granules (75 to 100 A. in diameter) and is, as such, a storage form of carbohydrate. The dispersed phase (non-aggregated granules) of glycogen is found within the sarcoplasm, primarily in the region of the Z line and A band. It is possible that in this form it may be readily used for energy purposes since the glycogen distribution and the location of extra-mitochondrial oxidative enzymatic activity is idential (1).

SUMMARY

1. PAS and lead hydroxide positive material, presumed to be glycogen because it is hydrolyzable by diastase and saliva, is localized in the intermyofibrillar spaces in the region of the I band. The intermyofibrillar glycogen, as determined by electron microscopy, appears to be an aggregated form (approximately 150 to 500 A. in diameter) of small granules 75 to 100 A. in size.

2. PAS and lead hydroxide positive material, presumed to be glycogen because it is hydrolyzable by diastase and saliva, is also localized in the myofibrillar sarcoplasm, principally in the A band and Z line. The particle size of the intramyofibrillar glycogen is approximately 75 to 100 A. in size. These are the dispersed form of the larger aggregates described above.

3. The combined use of light and electron microscopy techniques appear to reconcile earlier accounts of glycogen localization.

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FLUORESCENT STAIN FOR AMYLOID

An Easy Histological Differentiation Between Cutaneous Amyloidosis, Colloid Milium and Senile Elastosis[1]

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INTRODUCTION

Fluorescence microscopy is one of the newest tools utilized in histology and histochemistry. This field involves not only fluorescent antibody methods, but also the utilization of fluorescent stains to demonstrate certain tissue constituents (1). The best known techniques are acridine orange for nucleic acids (1), acridine orange and an iron-containing dye for "mucins" (2), and several fluorescent stains for lipids (3). The use of fluorochromes in skin histology and histochemistry has been limited. Jarrett described the use of various fluorochromes in normal skin (4) and in the differentiation of acantholytic cells (5).

Recently, Vassar and Culling (6) reported their results with the use of several fluorescent stains. In their hands, amyloid deposits in the kidney and skin fluoresced after staining with thioflavine T. They refer to a case in which primary amyloidosis with skin involvement was suspected, but staining of the skin biopsy with methyl violet and congo red gave very poor reactions. Thioflavine T staining of the skin sections gave a very strongly positive reaction, and subsequent studies confirmed the diagnosis of primary amyloidosis.

Because of this report, we undertook to try the use of this fluorochrome to demonstrate cutaneous amyloid deposits and differentiate it from colloid milium and severe senile elastosis. This differentiation is, at times, very difficult with the usual histological stains in general use.

METHODS

Sections that were utilized in this study were from four sources:

1. Sections of skin were obtained from the dermatopathology and surgical pathology laboratories of The Johns Hopkins Hospital. These included three cases of lichenoid amyloidosis, one of primary systemic and one of primary familial amyloidosis. Also two cases of colloid milium and three cases of severe senile elastosis were included. These

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tissues had been formalin-fixed, and the paraffin-embedded blocks were recut and stained as outlined below.

2. Formalin-fixed blocks of heart muscle obtained at necropsy from two cases of primary amyloidosis.

3. Zenker-fixed surgical biopsy specimens from the liver and stomach of a case of primary amyloidosis, and kidneys of two cases of secondary amyloidosis.

4. Fifty consecutive biopsy specimens received at the dermatopathology laboratory were also subjected to the same staining procedures. These biopsies showed the following histological features: eight were basal cell epitheliomas, six dermal melanocytic nevi, four verruca vulgaris, three each of epidermal papilloma and seborrheic keratosis, two each of lupus erythematosus, necrobiosis lipoidica, erythema multiforme, vasculitis and normal skin, and one each of psoriasis, lichen planus, scleroderma, junctional nevus, blue nevus, keratoacanthoma, senile keratosis, superficial epithelioma, metastatic carcinoma of breast, neurofibroma, prurigo nodularis, ichthyosis, dermatitis herpetiformis, acne keloidalis, alopecia areata, and pseudoxanthoma elasticum. This group served as control.

The following staining procedure was utilized:

1. Deparaffinize and bring to water in the usual manner.
2. Stain in 1 per cent aqueous solution of thioflavine T for three minutes.
3. Differentiate in 1 per cent acetic acid for ten minutes.
4. Dehydrate through graded alcohols and xylol.
5. Mount in Harleco Synthetic Resin-xylene Solution (H.S.R.)

All material was stained with hematoxylin and eosin, and the sections from groups 1 and 2 were also stained with toluidine blue, VanGieson, congo red, and crystal violet stains.

A Leitz-Wetzlar Ortholux microscope was used, utilizing a Phillips Mercury 150 watt high-pressure lamp. A UV filter (2mm. UG1) was used between the source and a dark field condenser, and an ocular filter (Euphos 2.5 mm.) was used for protection and photography.

RESULTS

Thioflavine T did not produce demonstrable staining of the tissues by gross examination or with illumination by the ordinary tungsten source. With the ultraviolet source, all of the nuclei and the hair shafts in the sections studied fluoresced with a bright yellow color. Almost all of the tissue appeared dull blue. Keratin was bright blue. Deposits of amyloid, however, fluoresced with a distinct and bright, light blue color.

In heart muscle, thioflavine T revealed heavy deposits of fluorescent material in the walls of all the medium and large sized vessels. Similar deposits were present between the muscle bundles, the latter being "squeezed". In the fat lobules, the amyloid was seen in a ring-like manner around the fat cells. (Fig. 1).

Hematoxylin and eosin staining of the skin from cases of amyloidosis and colloid milium revealed discrete clumps of amorphous, poorly stained material high in the papillary dermis. Similar clumps of amorphous material blending with basophilic fibers of elastic nature were seen in the

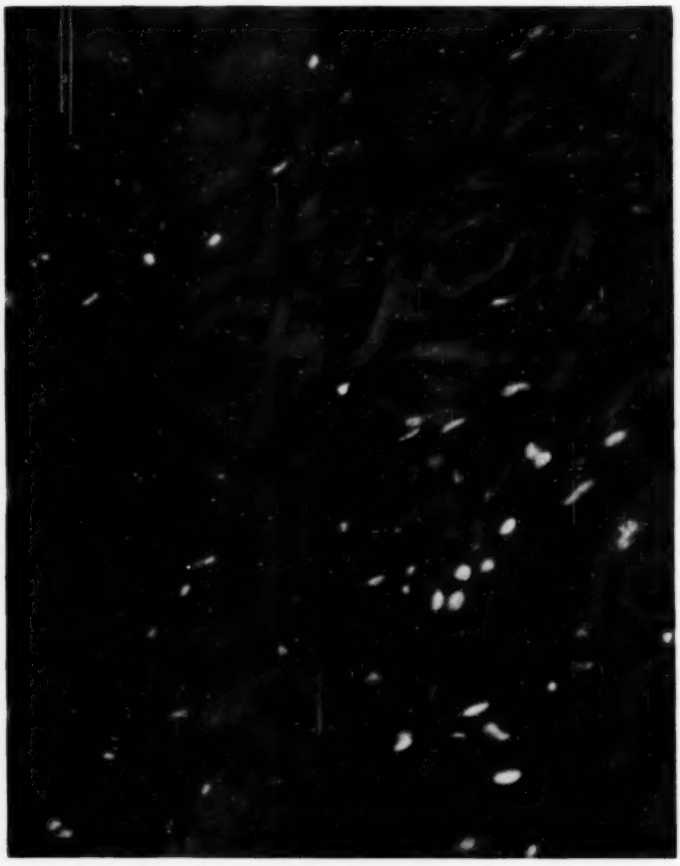

FIG. 1. Heart: Case of primary amyloidosis. Thioflavine T. Amyloid deposits seen as bright fluorescent bands around the myocardial bundles.

sections of senile elastosis. With this stain, a distinction between colloid milium and cutaneous amyloidosis was not possible. (Fig. 2).

Sections of colloid milium and amyloidosis stained with congo red, toluidine blue and VanGieson's stain were not dissimilar. Crystal violet staining of the altered tissue produced a brighter violet metachromasia in one case of amyloidosis than in the cases of milium, but the distinction was not sharp.

Thioflavine T staining produced brilliant fluorescence in the amyloid deposits. In primary amyloidosis, these deposits were seen in the form of rounded bundles in the papillary layer of the dermis as well as between the

FIG. 2A.

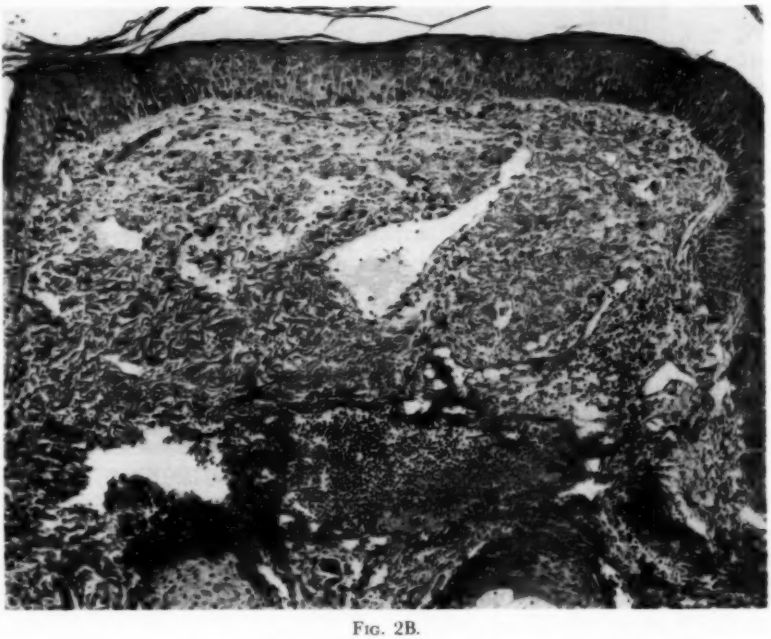

FIG. 2B.

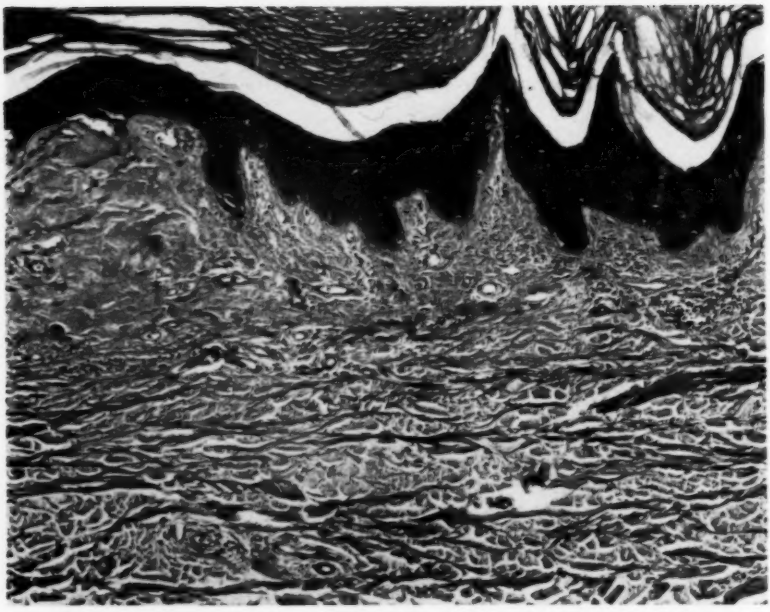

FIG. 2C

FIG. 2. Skin: Hematoxylin and eosin ×100. A. Colloid milium. B. Senile elastosis. C. Amyloidosis. Similar masses of amorphous material seen in the papillary portion of the dermis in the three conditions.

thick collagen bundles. Deposits were also present in the vessel walls. In the periphery of the fat cells of the subcutaneous tissue, "rings" of fluorescence were formed about the fat cells. There was mild deposition around the sweat glands. In lichenoid amyloidosis, the deposits were restricted mainly to the papillary areas of the dermis, and were seen as amorphous masses. (Fig. 3).

The fluorescence of the amyloid deposits was quite distinct and easily distinguishable and was not produced by any other part of the tissue. In particular, "deposits" of colloid milium and senile elastosis did not shown any fluorescence. (Fig. 4).

There was some variation in the brilliance of the fluorescence of the amyloid deposits in the different sections and tissues studied. The most brilliant fluorescence was seen in some of the cases of lichenoid amyloidosis. Zenker's-fixed tissues from the liver and stomach of a case of primary amyloidosis, and kidneys from the cases of secondary amyloidosis showed fluorescence of the amyloid deposits but of less brilliance than that seen in formalin-fixed tissues.

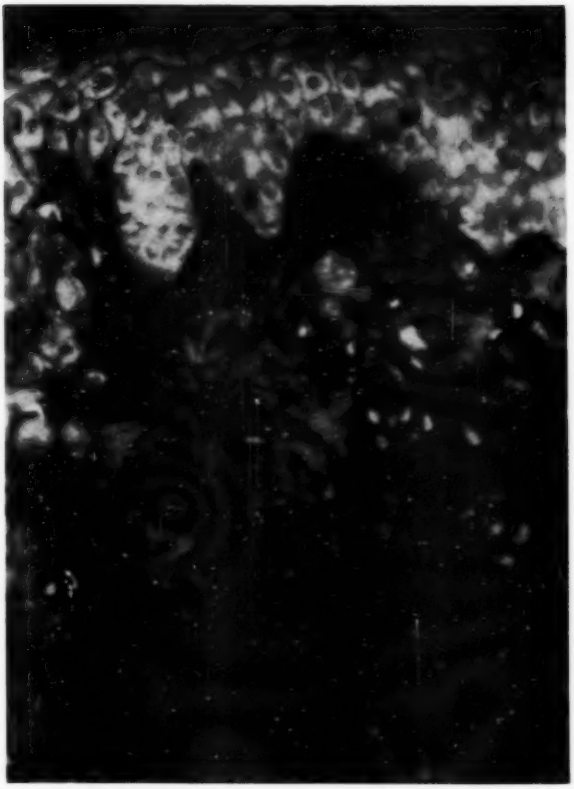

FIG. 3. Skin: Case of lichenoid amyloidosis. Thioflavine T. The epidermal and dermal nuclei fluorescence with a brilliant yellow color. In the dermis, except for the nuclei, all fluorescence is the bright blue of amyloid deposits.

The use of Apathy's medium (6, 7) for mounting did not prove as satisfactory as Harleco Synthetic Resin-xylene Solution (H.S.R.)

DISCUSSION

The chemical composition of amyloid has been the subject of recent studies. Vasquez and Dixon (8), using radioiodinated fluorescent antibodies, demonstrated that amyloid deposits contain high concentrations of gamma globulins and small amounts of albumin. Calkins and Cohen (9) showed that homogenates of amyloid-infiltrated livers and spleens contained proteins, carbohydrates, and a high water content. The carbohydrates in amyloid were in the form of hexosamine and free hexoses. The uronic acid content was less than 0.1 per cent, and the hydroxyproline con-

FIG. 4. Skin: Case of colloid milium. Thioflavine T. There is no fluorescence in the subepidermal areas except for the nuclear fluorescence.

tent was less than 1 per cent. Kaufman (10) analyzed the amyloid deposits in one case and found it to be made up of 76 per cent water, 12.5 per cent nitrogen, and 4.81 per cent carbohydrate. The uronic acid content was 0.45 per cent.

The complexity and variability in the chemical constituents may be responsible for the inconsistency in the usual staining reactions used. The low hexose and uronic acid content may explain the faint reaction with Periodic Acid Schiff Stain (11). This same PAS positive material may inhibit the compounds that stain metachromatically with toluidine blue, methyl violet and crystal violet (12). The variability of the reaction with VanGieson's stain and congo red is well known (13), especially in cases of primary amyloidosis (14, 15).

Because of the difficulty in the histological identification of amyloid

deposits in the skin and other tissues, skin pathologists have been faced with the problem of differentiating amyloidosis from other deposits or degenerative changes in the form of colloid milium and severe senile elastosis. With most of the stains in common usage, the amorphous masses within the papillary portion of the dermis may be similar in the three conditions (13). If deposits of amyloid are present in the deeper dermis as well, distinction from milium and elastosis may be possible inasmuch as in the latter two conditions the pathological changes are in the upper dermis only. This distinction, however, is lessened by the atypicality of staining reactions of primary amyloidosis (14, 15) which produces the deeper deposits.

In many instances, however, only papillary deposits of amyloid occur. The apparently selective fluorescence of this material with thioflavine T becomes of great importance in allowing a clear-cut diagnostic distinction. Tissues from cases of lichenoid amyloidosis, primary systemic amyloidosis, primary familial amyloidosis and secondary amyloidosis were stained and fluorescence was invariably present in the amyloid deposits and contrasted sharply with surrounding tissues. The variation in brilliance in the deposits may be related to fixation of the tissue or variation in concentration or in chemical structure of the amyloid. Autofluorescence of amyloid is known to occur, but is not prominent enough to be easily identifiable (8, 11). The ease of procedure and sharpness of definition of the deposits were considered to be factors of importance in selection of this technique for routine use in the laboratory.

SUMMARY

Thioflavine T, a fluorochrome, was used to show amyloid and was found to give excellent contrast of the deposits in the skin and viscera. In particular, the stain affords histologic differentiation between cutaneous amyloidosis, and colloid milium and senile elastosis.

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HEART FAILURE ASSOCIATED WITH UNUSUAL DEPOSITION OF GLYCOGEN IN THE MYOCARDIUM

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Glycogen storage disease of the heart is a metabolic disorder of infancy manifested by poor growth, cardiomegaly, hypotonia and death during the first year (1). A case of what may be an atypical form of the disease in a child who died in heart failure at fifteen years of age was reported by Antopol *et al.* (2). The autopsy of this child showed generalized cardiomegaly with normal valves and coronary circulation. The myocardium was pale and chemical studies performed twelve hours after death showed a glycogen content of 4 per cent. The liver and the kidneys were normal microscopically. Histologically, the myocardial fibers were distended with glycogen and there was also diffuse interstitial fibrosis. A sibling (not autopsied) showed a similar clinical course and died in cardiac failure at eleven years.

Two similar unusual cases of glycogen deposition and scarring in the heart have been seen in this hospital. Heart failure was prominent in both and in neither was any abnormality of the coronary circulation found to account for the myocardial fibrosis.

CASE REPORTS

Case 1: T.B. (B46872). A twelve year old white boy was referred to Harriet Lane Home Cardiac Clinic for "asthmatic bronchitis" which had begun when he was four years old. He had one to two annual attacks of wheezing, labored respiration, shortness of breath and cough. In symptom-free intervals, he was able to keep up with classmates in activities and school work. When he was seven years of age, his mother was told that he had an enlarged heart, but no special studies were carried out nor were his activities restricted. At the age of ten, dyspnea at rest and with exercise became apparent. Shortly before admission, he experienced two episodes of crushing precordial pain and dyspnea after exercise. He lost consciousness for several minutes with each attack.

Physical examination showed: Weight, 68 pounds; height, 56 inches; respirations, 28 per minute; pulse, 60 per minute; B.P. 110/78. Significant findings were limited to the cardiovascular system. The point of maximal impulse was in fifth intercostal space in the midclavicular line, and the heart was enlarged to percussion, rhythm was regular with slow rate and

no murmur was heard. The pulmonary second sound was louder than the aortic second sound; the first sound at the apex was loud and a distant third sound was heard. The lungs were clear to percussion and auscultation. The liver was felt 6 to 8 cm. below the right costal margin and was thought to be pulsatile.

Fluoroscopic examination: The heart was greatly enlarged; the pulmonary conus was convex. In the oblique views, the right ventricle extended to the anterior chest wall and the left ventricle cleared the spine. Barium swallow revealed a left aortic arch and marked left atrial enlargement.

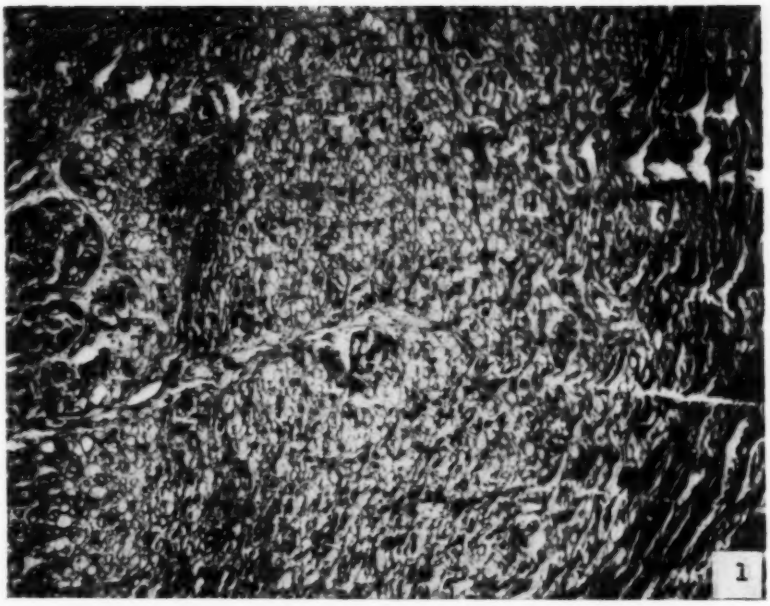
Laboratory findings: Hemoglobin 13.2 gm.; hematocrit, 46. Electrocardiogram showed balanced axis, right ventricular hypertrophy, incomplete right bundle branch block and left atrial enlargement. Cardiac catheterization revealed normal peripheral arterial saturation, severe pulmonary hypertension (mean pressure of 75 mm. Hg) and no evidence of an intracardiac shunt. An angiocardiogram demonstrated right auricular dilatation. The pulmonary artery was enlarged, the left atrium was visualized slowly because of its great size. The left ventricle and the aorta were visualized with difficulty.

Clinical Impression: The most likely diagnosis was thought to be mitral stenosis, with cor triatriatum a second possibility.

Course in hospital: Because of the severe pulmonary hypertension and left atrial enlargement with right ventricular hypertrophy a thoracotomy was performed. Pressures recorded across the mitral valve showed no gradient and the left atrium was then explored for a tumor. During this procedure, extreme hypotension developed followed by ventricular fibrillation. Despite cardiac massage and defibrillation the patient died on the operating table.

Autopsy (27863) performed three hours after death, was limited to the heart, lungs and liver. The heart weighed 270 grams (normal, 122 grams) with dilatation and hypertrophy of all chambers. The epicardium was discolored and roughened by surgical procedures and incisions had been made into the right auricular appendage, superior part of left atrium and left ventricle. All of the valves were normal; there were patches of endocardial thickening in the left ventricle. The foramen ovale was closed, the interventricular septum intact. The coronary arteries and venous return were normal. The myocardium was a translucent pale tan brown. The lungs were collapsed and airless. The liver was enlarged and showed prominent mottling.

Microscopically, the heart showed areas of extensive replacement of muscle fibers by connective tissue rich in collagen. In large adjacent zones (Fig. 1) and in foci distant from any scar (Fig. 2) fibers were swollen and vacuolated. The material lost from these fibers, was shown in periodic Schiff



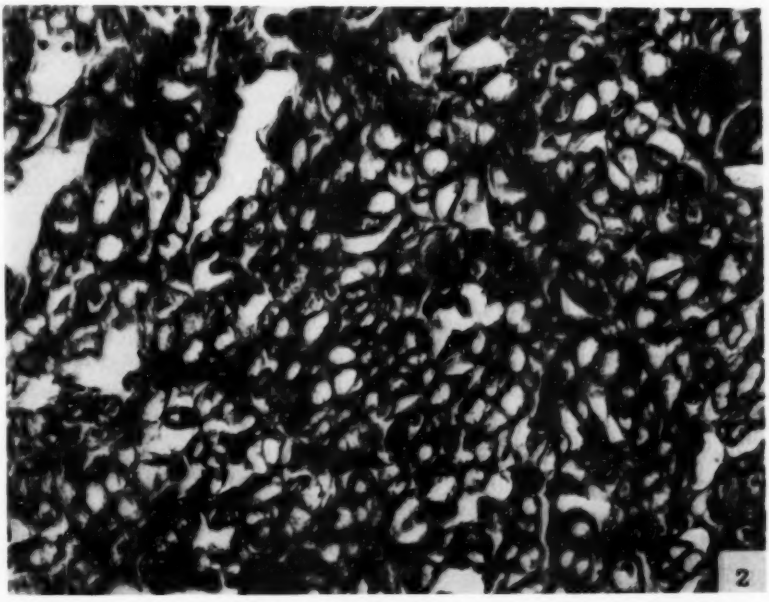


FIG. 1. Case 1 (Autopsy 27863): H & E stain of myocardium ×50, showing edge of dense collagenous scar with adjacent zone of glycogen-filled fibers.

FIG. 2. Same: H & E stain of myocardium ×160, showing area of glycogen-filled fibers distant from any scarring.

and Best carmine stains to be glycogen. There was no excessive glycogen in the liver which showed only severe chronic passive congestion with early central scarring. The lungs showed chronic passive congestion and atelectasis.

Anatomical Diagnosis: Focal glycogen storage in myocardium with scarring. Cardiac dilatation and hypertrophy. Chronic passive congestion, lungs. Cardiac cirrhosis. History of chronic "asthmatic bronchitis". History of pulmonary hypertension. Thoracotomy and cardiotomy wounds. History of cardiac arrest. Atelectasis.

Case 2: G.K. (A70767) An eleven year old white boy was referred to the Harriet Lane Cardiac Clinic with a history of a heart murmur detected at the age of three weeks. He had gained weight poorly and at six months of age, he had his first episode of paroxysmal tachycardia with a rate of 240 to 260 per minute. There was minimal response to digitalis. From that time until the age of seven years, the patient had one or two similar attacks each week; thereafter, he was free from attacks. A few weeks before his admission he developed paroxysmal nocturnal dyspnea and pedal edema.

Physical examination: Weight, 69 pounds; respiration, 36 per minute; pulse, 110 per minute; B.P. 105/90. He appeared thin and dyspneic. Lungs were clear to auscultation and percussion. The apex of the heart was felt in the fifth and sixth left interspaces in the anterior axillary line. A soft systolic murmur was heard over the entire precordium, loudest in the fourth left interspace. No diastolic murmur was heard. The rhythm was regular with a protodiastolic gallop at the apex. There was no accentuation of the second sound in the pulmonic area. The liver was enlarged to the umbilicus but did not pulsate. There was pitting edema of the extremities.

Fluoroscopic findings: The heart was enlarged to the left, the pulmonary conus was convex. In the left anterior oblique view the left ventricle did not clear the spine. There was great enlargement of the right auricle and the right ventricle. The lung fields were normal.

Laboratory findings: Hemoglobin 14. gm.; RBC 5,070,000; hematocrit 42. An electrocardiogram revealed left bundle branch and occasional extrasystole.

Clinical Diagnosis: In view of the history of arrhythmia, exercise intolerance and finally cardiac failure with gallop rhythm, minimal systolic murmur, large right auricle and right ventricle, the most likely diagnosis was thought to be Ebstein's malformation of the tricuspid valve.

Course in hospital: He was admitted to hospital and was digitalized. During an attempt at cardiac catheterization he developed ventricular fibrillation and died rapidly.

Autopsy (28161) was performed four hours after death. The heart weighed 300 grams (normal 116 grams) and all chambers were hypertrophied and dilated. The foramen ovale was closed and the interventricular septum was

intact. The central cusp of the tricuspid valve was shortened and fixed to the ventricular wall. The other valves were normal. The cardiac vasculature was entirely normal. However, patchy scarring was noted throughout the left ventricle, and there was diffuse endocardial thickening on the left side. No other gross lesions were seen.

Microscopically, the heart showed dense scars in the areas noted grossly. Confluent with them were zones of swollen myocardial fibers containing central vacuoles. Large groups of vacuolated fibers were prominent in subendocardial areas and many similar bundles of vacuolated fibers were completely independent of the scarring (Fig. 3–6). Best's carmine and periodic Schiff stains demonstrated that the contents of the vacuoles in the hematoxylin and eosin stains was glycogen. No glycogen was stainable in the other tissues. The lungs showed atelectasis and many "heart failure" cells.

Anatomical Diagnosis: Peculiar glycogen storage disease of the heart. Extensive endocardial and myocardial scarring. Cardiac hypertrophy. Malformation of tricuspid valve. History of cardiac massage; subepicardial hemorrhage. Atelectasis and chronic passive congestion, lungs.

DISCUSSION

In both of these cases of heart failure in children well past infancy, autopsy showed extensive deposits of glycogen and focal scars in the myocardium. The excess of glycogen deposition could conceivably be a result of the scarring or it might represent a primary metabolic defect. Wittels and Reinder (3) found excessive glycogen deposition in the myocardium adjacent to infarcts or secondary to anoxia from a variety of causes. In the present cases, however, there was no anemia, no abnormality of the coronary circulation, and no malformation that would lead to myocardial anoxia, necrosis and scarring. The distribution of the scarring is quite unlike that which follows a Fiedler's myocarditis. The endocardial fibrosis in the second case might be suggested as the primary lesion, but Kelly and Anderson (4) found no excess of glycogen in the congenital form of endocardial fibroelastosis, although in one typical case of infantile glycogen storage of the heart there was associated endocardial fibroelastosis. In a recent report of glycogenosis in 3 siblings (6), the authors reach the reverse conclusion, namely that endocardial fibroelastosis may be secondary to prolongation of glycogen abnormality. In one sibling dying at 3 months the endocardium was normal; in another sibling dying at 3 months there was minimal endocardial thickening; but in the sibling surviving to 10 months of age, there was severe endocardial fibroelastosis. The authors suggest that the absence of fibroelastosis in infants less than 3 months of age is further evidence that it developed in the older infant because of long

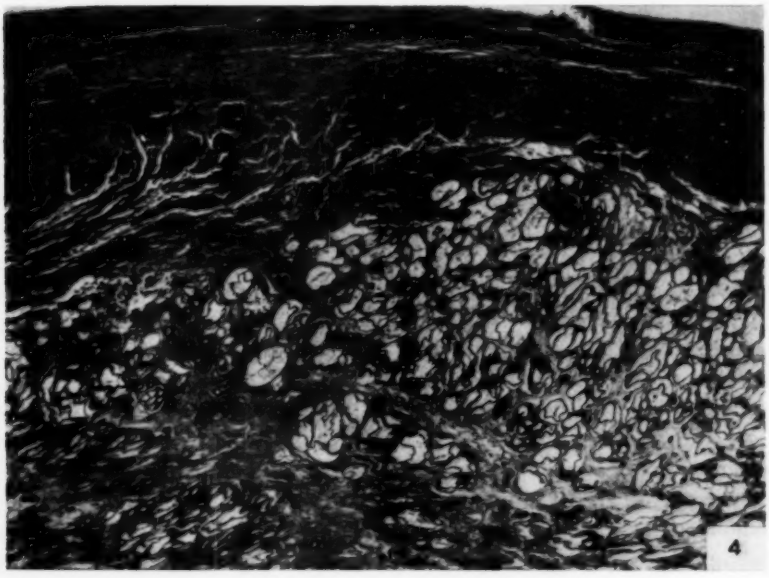
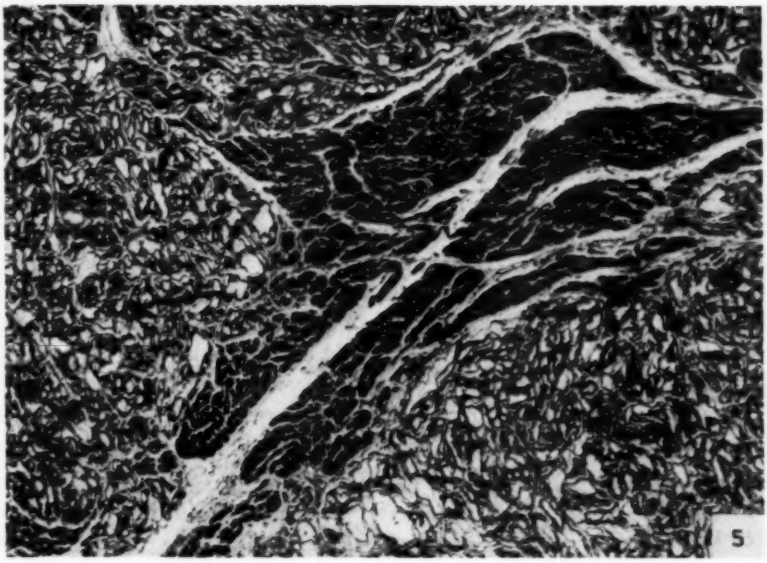


FIG. 3. Case 2 (Autopsy 21861): H & E stain of myocardium ×6.5; scanning view showing endocardial fibrosis (at top of photograph), beneath which is a wide vacuolated (glycogen-filled) subendocardial zone. Focal areas of scarring can be seen at the upper left and radiating through the center. The lower fields also contain glycogen vacuoles.

FIG. 4. Same ×50, showing endocardial fibrosis, and subendocardial scarring and vacuolization (glycogen).



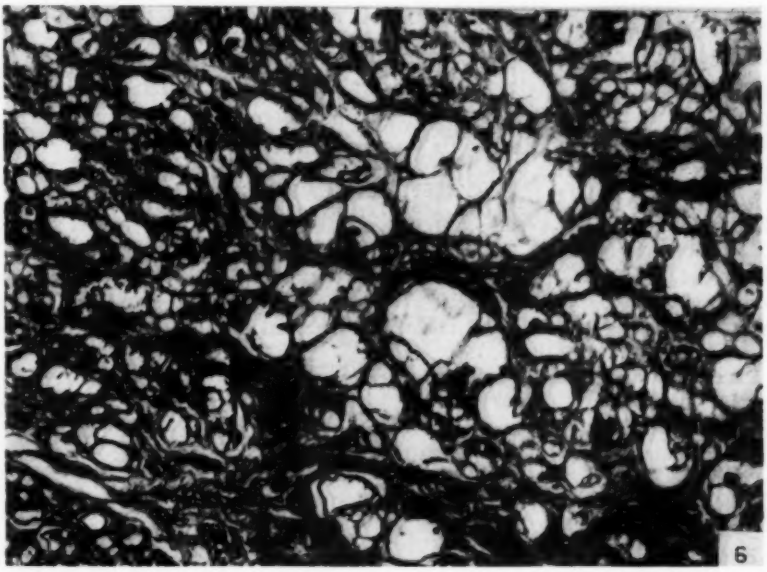


FIG. 5. Same ×50, showing focal scar with surrounding glycogen-filled myocardium (azo-carmine stain).

FIG. 6. Same ×160, showing great distension of fibers with glycogen causing their disruption. Compressed atrophic and scarred fibers can be seen in the center and lower portion of the field.

exposure of the myocardium to abnormally high levels of glycogen. "Fibroelastosis" secondary to other congenital cardiac abnormalities is not associated with abnormal deposition of glycogen (5).

The possibility that the abnormal glycogen deposition could be due to a primary metabolic defect must be considered although this is classically associated with a defective glycogen metabolism in other tissues. Antopol *et al.* (2) postulated the existence of an atypical form of cardiac glycogen disease with the abnormality limited to the heart. Presumably, gradual overloading of groups of fibers led to necrosis, scarring and myocardial insufficiency. The microscopic findings in the present cases support this explanation, all stages of glycogen storage, myocardial disruption, atrophy, necrosis and scarring being present in both hearts. Furthermore, there was good clinical evidence of longstanding cardiac disease. It seems probable that glycogen storage abnormality limited to the heart was the primary lesion in both cases. This variant is characterized by limitation of the glycogen deposition to the heart and much longer duration of life than is seen in the typical infantile form of glycogen storage disease.

SUMMARY

The clinical course and autopsy findings in two boys dying at twelve and eleven years of age are described. In both, the basic cardiac lesion was unusual focal myocardial glycogen storage, associated with extensive scarring. In one case, endocardial fibrosis was also present.

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IN VITRO AND IN VIVO SUSCEPTIBILITY OF PROTEUS SPECIES TO THE ACTION OF CERTAIN ANTIMICROBIAL DRUGS[1]

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Proteus species are frequently cultured from the urine of patients with chronic and anatomically-complicated infections of the urinary tract (1). These organisms are also capable of causing severe systemic infections, including acute pyelonephritis, bacteremia, cellulitis, abscesses, endocarditis, mastoiditis, or meningitis (2 to 5). Despite recent advances in antimicrobial therapy, the treatment of Proteus infections is in general unsatisfactory. This may be related, at least in part, to the relative resistance of these bacteria to the action of the commonly-used antibiotics.

The purpose of the present report is to describe the results of *in vitro* sensitivity tests of 127 recently-isolated strains of Proteus against seven antibiotics. In addition, a number of case reports are included to illustrate the high degree of correlation between *in vitro* sensitivity and *in vivo* response to antimicrobial drugs, and to emphasize the potential usefulness of penicillin in the treatment of certain Proteus infections.

MATERIALS AND METHODS

The strains of Proteus bacilli were obtained from the Bacteriology Laboratories of the Johns Hopkins Hospital through the courtesy of Mrs. Isabelle G. Schaub. Proteus species were identified as described by Schaub and associates (6), and only strains present in urine in numbers of 100,000 or more per ml. were tested (1).

The present study includes 88 strains of *Proteus mirabilis* (indole-negative strains) and 39 strains of *P. rettgeri*, *P. vulgaris*, and *P. morgani* (indole-positive strains). Although these numbers do not reflect the precise incidence of isolation of indole-negative and indole-positive species from urine, they are in close agreement with data gathered at a number of other institutions (7 to 12) indicating that the majority (70 to 90 per cent) of Proteus strains isolated from clinical infections is identified as *P. mirabilis*.

Tests for antibiotic susceptibility were performed utilizing the tube dilution technique previously described and both bacteriostatic and bactericidal end-points were obtained (13). All strains were tested against potassium penicillin G, tetracycline hydrochloride,

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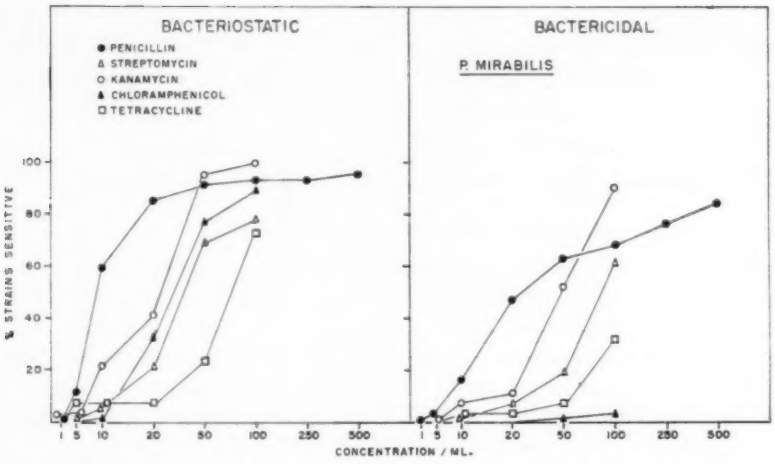

FIG. 1. Susceptibility of 88 strains of *P. mirabilis* to the action of five antibiotics. The concentration of penicillin is expressed as units per ml., and the concentrations of the other antimicrobials as microgm. per ml.

chloramphenicol, polymyxin B sulfate, streptomycin sulfate, kanamycin sulfate and sodium colistin methanesulfonate.

RESULTS

In Vitro Sensitivity. Striking differences in antibiotic sensitivity were observed between strains of *P. mirabilis* (Fig. 1) and strains of the other three Proteus species (Fig. 2). In general, strains of *P. mirabilis* were much more sensitive to the action of penicillin, streptomycin, chloramphenicol or tetracycline than were strains of Proteus that produce indole (*P. rettgeri*, *P. vulgaris*, *P. morgani*).

Penicillin was the most active antibiotic against *P. mirabilis*. Seventy-five of the 88 strains were inhibited by penicillin in a concentration of 20 units or less per ml. and 80 strains were inhibited by 50 units per ml. In most instances, the bactericidal concentrations of penicillin were 2 to 4 times the amount required for bacteriostasis. In contrast to the effect of penicillin against *P. mirabilis*, only 1 of 39 indole-positive strains of Proteus was inhibited by 100 units per ml. of penicillin.

Kanamycin showed approximately equal activity against the four species of Proteus. Sixty-four of the 127 strains were inhibited by kanamycin in a concentration of 20 microgm. per ml. and 122 strains were inhibited by 50 microgm. per ml. Bacteriostatic and bactericidal end-points with kanamycin were identical for one-third of the strains. With the other strains, 2

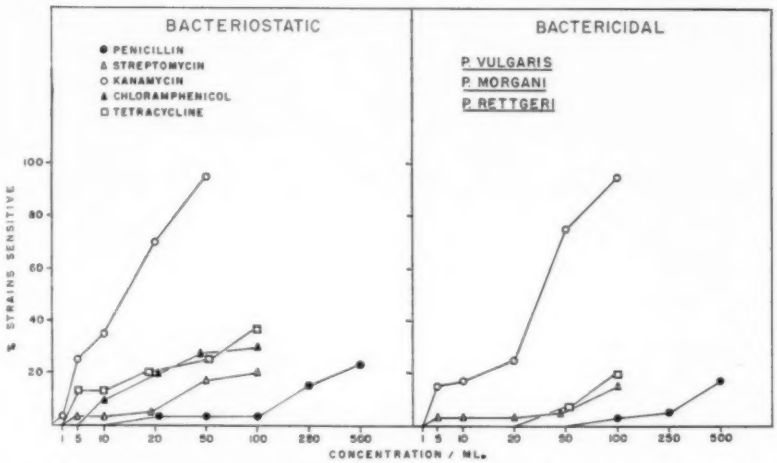

FIG. 2. Susceptibility of 39 strains of *P. vulgaris*, *P. morgani* and *P. rettgeri* to the action of five antibiotics. The concentration of penicillin is expressed as units per ml., and the concentrations of the other antimicrobials as microgm. per ml.

to 4 times the concentration of kanamycin were required for bactericidal effect than was necessary for bacteriostasis.

Although chloramphenicol and tetracycline inhibited growth of *P. mirabilis* more effectively than the other species of Proteus, this effect was apparent only with high concentrations of these drugs. Tetracycline displayed unimpressive bactericidal action, and chloramphenicol in a concentration of 100 microgm. per ml. was lethal for only 3 of 127 strains of Proteus.

Polymyxin B sulfate and colistin methanesulfonate in concentrations of 100 microgm. per ml. were totally inactive against the 127 strains of Proteus.

Results of Therapy. As illustrated in the following case reports, a high degree of correlation was observed between the sensitivity of individual Proteus strains to an antibiotic and the response of the patient to antimicrobial therapy. Five of the patients to be described had *P. mirabilis* infections and were treated with penicillin alone.

Case 1. D.F., a 29 year old housewife, was hospitalized because of chills, fever, and dysuria beginning 24 hours after cystoscopy for retrograde pyelography. She gave a history of recurrent episodes of cystitis and pyelonephritis for 2 years. Previous urologic evaluation which included an intravenous pyelogram revealed no abnormality of the urinary tract.

Quantitative urine culture revealed more than 1×10^6 *P. mirabilis* per ml. of urine. Growth of this organism was inhibited by penicillin in a concentration of 20 units per ml.

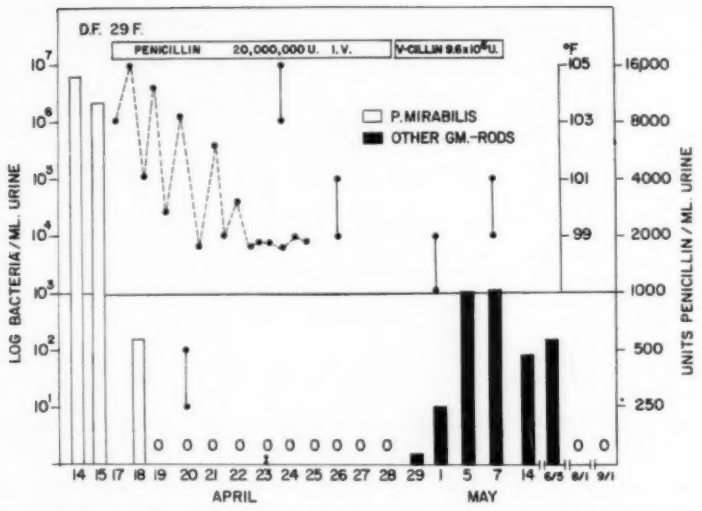

FIG. 3. Case 1: Temperature record, results of quantitative urine cultures and blood and urine levels of penicillin in a patient with *P. mirabilis* infection of urinary tract.

but a bactericidal effect was not observed with 250 units per ml., the highest concentration tested. Blood was sterile on culture.

As shown in Figure 3, during administration of 20×10^6 units of penicillin intravenously per day, a progressive decrease in fever occurred, symptoms related to urinary tract infection subsided, and the number of *P. mirabilis* in the urine decreased sharply. After 10 days of intravenous penicillin, potassium penicillin-V[4], 9.6×10^6 units per day by the oral route, was given for an additional 10 day period. During therapy with oral penicillin a variety of Gram-negative bacilli were cultured from the urine, but always in numbers of 10^3 or less per ml. There has been no clinical or bacteriologic evidence of recurrence of infection during a follow-up period of 15 months following completion of penicillin therapy.

The concentration of penicillin in urine or blood was determined a number of times while the patient was receiving penicillin (Fig. 3). During intravenous penicillin therapy, serum levels ranged from 64 to 128 units per ml., and urine concentrations usually exceeded 250 units per ml. Serum levels were not measured during therapy with penicillin-V. However, during this period several specimens of urine collected at random were found to contain 1022 or more units of penicillin per ml.

Comment: This patient had a moderately severe systemic reaction secondary to infection of the urinary tract with *P. mirabilis*. She responded promptly to treatment with penicillin in large doses and there has been no recurrence.

Case 2. C.C., a 56 year old man, was hospitalized for treatment of a *P. mirabilis* infection of the urinary tract. He gave a history of renal colic recurring 3 to 4 times each year

[4] V-cillin, made available through the courtesy of Dr. R. E. Griffith, Eli Lilly Laboratories, Indianapolis, Ind.

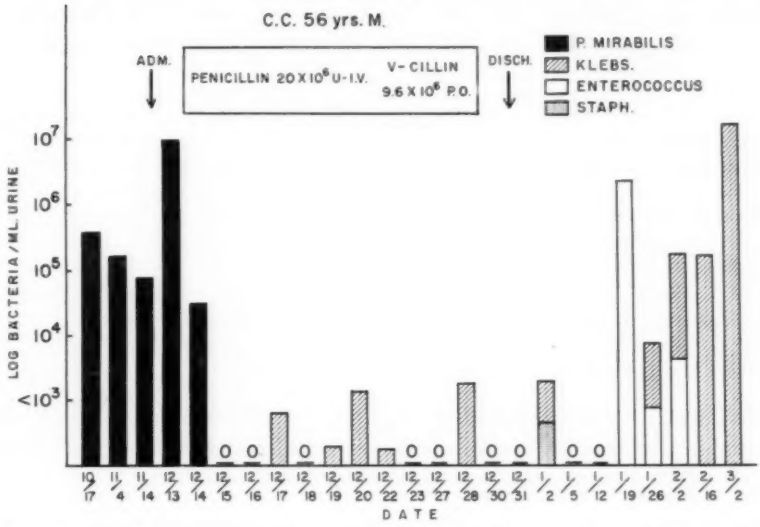

FIG. 4. Case 2: Results of quantitative urine cultures in a patient with *P. mirabilis* infection of urinary tract treated with penicillin.

for 15 to 20 years. A number of these episodes had been associated with dysuria, flank pain, chills, and fever, and many attacks were characterized by the passage of stones from the urethra. Eight years before admission he had had a left nephrectomy.

Extensive studies failed to reveal an underlying process other than urinary tract infection that might account for recurrent stone formation. Chemical analysis of the stones showed that they were composed of magnesium ammonium phosphate. The concentration of blood urea nitrogen was normal.

Quantitative urine cultures repeated on several occasions during the two months prior to hospitalization and those obtained after admission showed more than 1×10^5 *P. mirabilis* per ml. of urine. This organism was markedly resistant to tetracycline, chloramphenicol, streptomycin, and kanamycin, but was killed by 50 units per ml. of penicillin.

As shown in Figure 4, the patient was given 20×10^6 units of potassium penicillin G per day intravenously for 7 days, and then 9.6×10^6 units of potassium penicillin-V orally for an additional 7 days. Proteus bacilli were not cultured from the urine during therapy or during a subsequent follow-up period of 1 year. However, 1 month after penicillin was discontinued Klebsiella in large numbers appeared in the urine and persisted during the follow-up period. He has had no subsequent episodes of fever, flank pain, or renal colic.

Comment: Following administration of penicillin *P. mirabilis* disappeared from the urine of this patient with chronic Proteus infection and recurrent renal calculi. However, one month after penicillin therapy, Klebsiella in large numbers appeared in the urine. In this connection the observation that calculi develop in the urinary tracts of experimental animals with Proteus pyelonephritis is of interest (14 to 16). Although no causal relationship can be established, it is noteworthy that this patient has had no

episodes of renal colic after the Proteus infection was controlled and no further enlargement of the stones has been determined roentgenographically.

Case 3. A.B., a 41 year old Negro female with diabetes mellitus, diabetic neuropathy, and an atonic bladder, was hospitalized because of gangrene of the distal portion of one lower extremity. She gave a history of numerous catheterizations. Three separate specimens of urine contained large numbers of pus cells and *P. mirabilis* in numbers of 2×10^7 to 2×10^8 per ml. This organism was resistant to 100 microgm. per ml. of tetracycline, chloramphenicol, or streptomycin, and to 50 microgm. per ml. of kanamycin, but was killed by 20 units per ml. of penicillin.

She received 20×10^6 units of penicillin intravenously per day for 10 days, and then 9.6×10^6 units of penicillin-V orally for an additional 7 days. The number of Proteus bacilli per ml. of urine decreased from 2×10^8 to 4×10^3 after 24 hours of therapy and Proteus was not cultured from subsequent specimens of urine.

Comment: Although penicillin apparently controlled the *P. mirabilis* infection in this patient with neurogenic bladder dysfunction, reinfection with Proteus or another microorganism is almost inevitable.

Case 4. M.J., a 70 year old woman with diabetes mellitus and hypertension, was hospitalized because of subarachnoid hemorrhage. She developed a urinary tract infection with *P. mirabilis* shortly after an indwelling catheter was inserted. Proteus bacilli were eliminated from the urine during therapy with 20×10^6 units of penicillin intravenously per day for 10 days. Within 48 hours after beginning therapy with penicillin, Klebsiella in large numbers were cultured from the urine.

Comment: *P. mirabilis* was eliminated from the urine of a patient with an indwelling catheter. It is not surprising that this organism was replaced by a penicillin-resistant Klebsiella within 48 hours.

Case 5. D.G. was a 33 year old white female with disseminated lupus erythematosus, chronic pyelonephritis and bilateral staghorn calculi. During the 10 months prior to death she was hospitalized on 4 occasions because of fever, flank pain, and dysuria related to *P. mirabilis* infection of the urinary tract. The Proteus was highly resistant to the commonly-used antibiotics except penicillin which exerted a bactericidal effect in a concentration of 10 units per ml. On each of the four admissions she was treated with potassium penicillin G, $10–20 \times 10^6$ units intravenously per day, or potassium penicillin-V, 9.6×10^6 units orally per day. On each occasion, Proteus bacilli promptly disappeared from the urine and were replaced by Candida species in large numbers. As long as the patient was receiving penicillin, Proteus was not cultured from the urine, but within 1 to 6 weeks after the drug was discontinued, *P. mirabilis* reappeared in large numbers and Candida could no longer be isolated. The sensitivity of the Proteus to penicillin did not change over this period of 10 months.

Comment: Despite prolonged therapy it was not possible to eradicate *P. mirabilis* from the urinary tract of this patient with lupus erythematosus and staghorn calculi. It is of interest that the patient developed dysuria, fever and flank pain when significant Proteus infection was present but

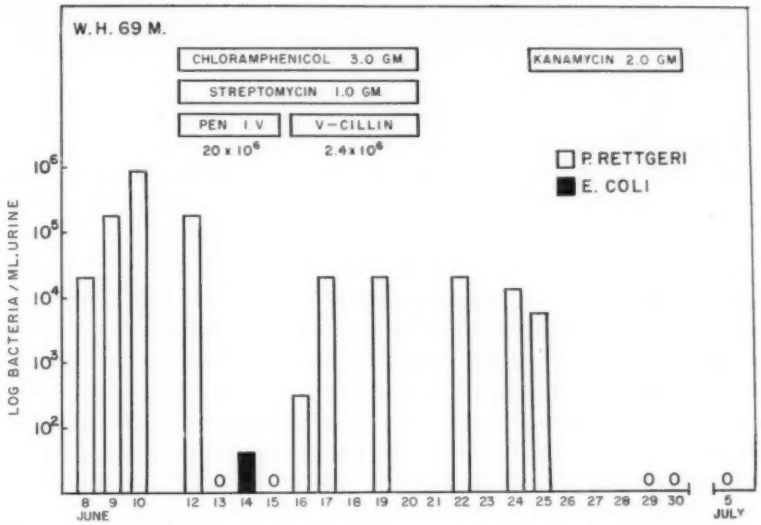

FIG. 5. Case 6: Effect of antimicrobial therapy on the number of bacteria in the urine of a patient with *P. rettgeri* infection.

experienced almost no symptoms referable to the urinary tract when Candida was the predominant organism.

Case 6. W.H., a 69 year old man, was admitted for treatment of a chronic infection of the urinary tract. He had previously undergone transurethral prostatectomy with some improvement in symptoms of obstructive uropathy. However, signs of urinary tract infection persisted and *P. rettgeri* in large numbers was cultured from the urine. This organism was resistant to more than 500 units per ml. of penicillin, and to more than 100 microgm. per ml. of streptomycin or chloramphenicol. As shown in Figure 5, administration of chloramphenicol, streptomycin and penicillin in large amounts resulted in transiently negative urine cultures. Nevertheless, *P. rettgeri* reappeared in the urine during therapy with these drugs. The *P. rettgeri* was killed *in vitro* by 5 microgm. per ml. of kanamycin, and rapidly disappeared from the urine during therapy with this drug. Urine cultures remained sterile during a 2 month follow-up period.

Comment: The urinary tract infection in this patient did not respond to therapy with 3 antibiotics to which the causative organism was shown to be highly resistant, but rapidly cleared after administration of kanamycin which was shown to be highly active *in vitro*.

Case 7. J.Z., a 71 year old man, was hospitalized because of multiple fractures sustained in an automobile accident. He experienced difficulty voiding and was catheterized. Subsequently he developed dysuria and pyuria, and *P. rettgeri* in numbers of 5×10^4 to 6×10^6 per ml. was cultured from four separate specimens of urine. This organism was resistant to 500 units per ml. of penicillin and 100 microgm. per ml. of tetracycline, chloramphenicol

or streptomycin. Kanamycin was bacteriostatic in a concentration of 1 microgm. per ml. and was bactericidal in a concentration of 5 microgm. per ml. The blood urea nitrogen was within normal limits.

Because of an error in prescribing the drug, he received kanamycin, 1 gm. orally every 12 hours for 3 days. Within 24 hours there was a marked reduction in the number of Proteus bacilli in the urine and thereafter daily urine cultures were sterile. After kanamycin had been administered orally for four days the error was discovered and subsequently the drug was given intramuscularly in doses of 0.5 gm. every 12 hours. There was no recurrence of bacteruria and pyuria after kanamycin was discontinued.

Comment: *P. rettgeri* could not be cultured from the urine of this patient after 24 hours of therapy with kanamycin given by the oral route. Kanamycin is poorly absorbed from the gastrointestinal tract (17), and this route of administration is not recommended for the treatment of systemic infections. However, in patients receiving a single dose of kanamycin orally an average of 0.7 per cent of the dose can be recovered from the urine in 24 hours (18). The present case is included to illustrate the prompt bacteriologic response of a susceptible microorganism to what were probably very small amounts of kanamycin.

Case 8. W.H., a 71 year old man with a history of bilateral nephrolithiasis and pyelonephritis, was hospitalized because of troublesome dysuria and frequency, and to rule out the possibility of parathyroid adenoma. He had had multiple courses of antibiotic therapy in the past. *P. rettgeri* and Klebsiella species in large numbers were cultured from the urine. The Proteus was resistant to 500 units per ml. of penicillin, and to 50 microgm. per ml. of tetracycline, chloramphenicol, and streptomycin, but was killed by 5 microgm. per ml. of kanamycin. The latter drug in a concentration of 1 microgm. per ml. was also bactericidal for the Klebsiella species. He received kanamycin, 2 gm. per day for 7 days, with prompt subsidence of dysuria and pyuria, and disappearance of bacteruria. However, urine culture performed 8 days after cessation of therapy with kanamycin revealed 156 *P. rettgeri* per ml. This organism exhibited the same antibiotic sensitivity pattern as the Proteus isolated prior to kanamycin therapy. Eighteen days after kanamycin had been discontinued the number of *P. rettgeri* had increased to 6×10^3 per ml., and 37 days after antimicrobial therapy the bacterial count was 2×10^5 per ml. Proteus continued to be present in large numbers during a follow-up period of 10 months. However, the patient was entirely asymptomatic during that time.

Comment: This case probably represents a treatment failure with kanamycin, although reinfection cannot be excluded. It is of interest that the organism which reappeared in the urine was not resistant to kanamycin.

Case 9. J.K., a 50 year old woman with severe hepatic cirrhosis, developed a *P. mirabilis* infection of the urinary tract after catheterization of the bladder. This organism tested by the disk method was found to be markedly sensitive to nitrofurantoin. By the tube dilution technique, streptomycin in a concentration of 50 microgm. per ml. was bactericidal, and chloramphenicol in a concentration of 20 microgm. per ml. was bacteriostatic, but exerted no bactericidal effect. The number of Proteus bacilli in the urine did not decrease during therapy with nitrofurantoin, 400 mg. orally per day, or with streptomycin, 2 gm. intramuscularly per day. After these drugs were discontinued, chloramphenicol in doses of 3 gm.

orally per day was given. Proteus bacilli were not cultured from urine for 3 days after chloramphenicol was started but thereafter were isolated in large numbers despite continuation of drug.

Comment: This patient was treated with antimicrobials shown to be relatively inactive *in vitro* against the causative organism. Bacteruria persisted although transient reduction in the number of organisms cultured occurred during administration of chloramphenicol.

DISCUSSION

In 1954, Potee, Wright and Finland (7) showed that the sensitivity of *P. mirabilis* to certain antibiotics was quite different from the sensitivity of the other three Proteus species. *P. mirabilis* strains were relatively susceptible to the action of penicillin, bacteriostatic concentrations ranging from 12.5 to 200 microgm. per ml., while the other three species were almost entirely resistant to penicillin even in concentrations greater than 200 microgm. per ml. Previous observations (19 to 24) had shown that the growth of certain Proteus strains was inhibited by penicillin but the correlation of species with antibiotic susceptibility was not recognized until the studies of Potee and associates.

The results of the present study as well as those recently described by Chiu and Hoeprich (8) demonstrate that the relative sensitivity of *P. mirabilis* to penicillin has not changed appreciably since the report of Potee *et al.* (7) 5 years ago. Eighty-five per cent of strains of *P. mirabilis* was inhibited by 20 units per ml. of penicillin and 90 per cent by 50 units per ml., a concentration readily attained in the blood stream during administration of 10 or more million units per day.

It is not surprising that kanamycin was found to have considerable activity *in vitro* against the four Proteus species. Earlier studies (7) showed that the majority of strains of Proteus is inhibited by neomycin and the spectra of activity of neomycin and kanamycin are quite similar (25).

In the present study strains of *P. mirabilis* also appeared to be more susceptible to the action of streptomycin, chloramphenicol and tetracycline than were the indole-producing strains. This is in contrast to the results of Potee *et al.* (7) who found the majority of strains of *P. rettgeri*, *P. morgani* *P. vulgaris* susceptible to tetracyclines.

The antibacterial activity of chloramphenicol deserves special comment. We, as well as others (7), have observed that growth of the majority of strains of *P. mirabilis* was inhibited by this agent in concentration of 50 microgm. per ml. However, if one distinguishes between bacteriostatic and bactericidal activities of the various drugs, as was done in this study, it becomes apparent that chloramphenicol even in a concentration of 100

microgm. per ml. exerts practically no bactericidal action against Proteus species. Only one of the 127 strains was killed by this drug.

Although penicillin has often been mentioned as a potentially useful agent for treatment of infections caused by *P. mirabilis*, there are relatively few reports documenting its clinical effectivenss. The results of the limited detailed clinical and bacteriologic observations described in the present report clearly indicate that penicillin in large doses is effective in eliminating *P. mirabilis* from the infected urinary tract. The doses of penicillin used were probably larger than necessary and additional observations are indicated to define optimal dosage schedule. It is clear, however, that small doses of penicillin are inadequate. Waisbren and Carr (24) obtained a satisfactory bacteriologic response in only 3 of 15 patients with Proteus urinary-tract infection treated with 600,000 units of penicillin per day, a dosage unlikely to yield tissue levels at which growth of Proteus species is inhibited.

We have also observed a number of patients with severe Proteus infections and bacteremia treated with large doses of penicillin. However, it has been impossible to evaluate the effect of penicillin because almost all patients received one or two additional antimicrobial drugs. In 1947 Hutchinson and Randall (26) described a patient with Proteus osteomyelitis and bacteremia who showed an initial response to therapy with penicillin alone but who relapsed after therapy was discontinued because penicillin was not available. In addition, Waisbren (27) and Holloway and Scott (10) each reported one case of systemic Proteus infection with bacteremia cured by administration of penicillin in doses exceeding 14 million units per day.

The selection of antimicrobials for therapy of Proteus infections should be based whenever possible on properly performed sensitivity tests. However, it may be necessary to initiate therapy before the results of sensitivity tests are available, or it may be impossible to obtain sensitivity tests. In this event, especially in patients with severe infections, the use of penicillin in dosage of 10 million or more units per day, or kanamycin in dosage of 2 grams daily should be considered. Because the usefulness of penicillin is limited to infections with *P. mirabilis*, it is important to distinguish these strains from other Proteus species (6). Since kanamycin may produce damage to the auditory portion of the eighth cranial nerve, particularly in patients with impaired renal function, it should be reserved only for critically ill patients or serious infections that have not responded to other antimicrobials.

SUMMARY

The sensitivity of 127 strains of Proteus bacilli isolated from patients with urinary tract infections was determined against penicillin, tetracy-

cline, chloramphenicol, streptomycin, polymyxin B, colistin, and kanamycin. In general, strains of *P. mirabilis* were more susceptible to the action of penicillin, tetracycline, chloramphenicol and streptomycin than were strains of *P. rettgeri*, *P. morgani* or *P. vulgaris*. Penicillin was the most active drug against *P. mirabilis* and kanamycin was the most effective agent against the other species. Polymyxin B and colistin did not inhibit bacterial growth in the concentrations employed.

Penicillin in large doses or kanamycin was effective in eliminating Proteus species from the urine of patients with infections of the urinary tract. A high degree of correlation was observed between the *in vitro* sensitivity of an organism to an antibiotic and the capacity of that agent to eliminate the organism from the urinary tract.

ACKNOWLEDGMENT

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SURVIVAL OF PLATELETS TRANSFUSED INTO THROMBOCYTOPENIC RECIPIENTS[1]

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INTRODUCTION

Hemorrhagic phenomena due to thrombocytopenia can be controlled by platelet transfusions providing the infused platelets continue to circulate in sufficient numbers (1 to 4). The survival of transfused platelets has been demonstrated by an increase of the recipients' platelet levels or by the use of platelets labelled *in vitro* or *in vivo* with radioactive substances (5 to 10). The post-transfusion survival of platelets has been shown to be adversely affected by various *in vitro* manipulations of the platelets (3, 8, 11) and by antiplatelet factors in the recipient (11 to 14).

In the present studies, some of the factors that influence the viability of platelets were determined by measuring the ability of platelets to circulate when transfused into thrombocytopenic recipients. Most of the studies were performed in dogs rendered thrombocytopenic by exposure to whole body radiation. This experimental model was used because the thrombocytopenia that follows whole body radiation has been shown to be due to lack of platelet production (15). In an additional study, platelet viability was determined using an animal rendered thrombocytopenic by an injection of rabbit anti-dog platelet serum (16).

MATERIALS AND METHODS

Donor blood was obtained from adult mongrel dogs that had not been transfused and that weighed more than 18 kg. Saline and protein crossmatch procedures were performed on all bloods prior to transfusion. Donor dogs were anesthetized with pentobarbital sodium and were bled approximately 500 ml. by femoral artery puncture using 15 or 17 gauge Arquad® coated needles and disposable plastic tubing. Phlebotomies were completed within 5 minutes. The blood was collected either in plastic bags[2] containing 75 ml. of acid-citrate-dextrose (ACD) solution Formula A or in uncoated, standard glass vacuum bottles containing 120 ml. of ACD solution Formula B. No special precautions were taken to prevent foaming in the vacuum bottles. Transfusions of fresh blood were performed using blood that had not been refrigerated and that had been collected within one hour of transfusion. Stored blood was kept at 4°C.

[1] This work was carried out under Contract AT(30-1)1208 between the Atomic Energy Commission and The Johns Hopkins University, and was supported in part by research grants from the Division of Grants and Fellowships of the National Institutes of Health and from the Daland Fund of the American Philosophical Society. An abstract of this work was published in Fed. Proc., 1960, **19:** 64.

[2] Kindly supplied by Fenwal Laboratories, Inc., Framingham, Mass.

Adult mongrel dogs that had not been previously transfused and that weighed from 8 to 18 kg. were used as recipients. Blood volumes of recipients were estimated as 9 per cent of body weight (17). Recipients were anesthetized with pentobarbital sodium for the period of transfusion. Transfusions were performed using Fenwal® plastic recipient sets which contained a filter. Exchange transfusions were performed in increments of 500 ml. by alternately bleeding the recipient by femoral artery puncture and transfusing donor blood until 2,200 to 2,625 ml. of blood had been infused and a like amount removed. Cross-transfusions were performed in increments of 500 ml. by repeated, simultaneous bleeding of two dogs by femoral artery punctures and immediately infusing the blood into the other animal until 2,725 to 2,900 ml. of blood had been infused. With all of the techniques of transfusion, the entire procedure was completed within 2 hours and 1 to 3 ml. of a 10 per cent solution of calcium chloride was administered intravenously after infusion of each 500 ml. of blood.

Seven recipient dogs received 500r whole body radiation and two received 450r. The animals were anesthetized with pentobarbital sodium for the period of irradiation. Radiation was delivered by a Keleket constant potential machine operating at 250 KVP, 15 ma. and filtered with 0.5 mm. copper and 1.0 mm. aluminum. One dog that was not irradiated was given antiplatelet serum[3] prepared by injecting dog platelets into rabbits (16).

Platelet counts were performed on venous blood by the phase contrast technique of Brecher and Cronkite (18). Platelet counts of donor blood at the time of transfusion were performed on blood that had passed through the recipient set and filter. Hematocrit values were determined by a microtechnique (19). Leukocytes were counted in blood diluted with 0.1 N. hydrochloric acid. Studies of blood coagulation were performed using previously described techniques (20).

RESULTS

The degree of fall of the platelets, leukocytes and hematocrit values of the dogs which received 450 to 500r whole body radiation were similar to those described previously by many investigators.

Five dogs (602, 603, 604, 605 and 608) were given 500r whole body radiation. The platelet level in each animal had fallen to $< 20{,}000$ per cu. mm. by the 8th to 11th day after irradiation. Each dog then was transfused with blood collected in plastic bags and no phlebotomies were performed on the recipients. Four dogs (602, 603, 604 and 605) were given 1,000 ml. of blood and one (602) exhibited signs of pulmonary edema at the end of the infusion. Dog 608 received only 900 ml. of blood because signs of pulmonary edema developed after infusion of this amount. Dog 608 received fresh blood. The other dogs received blood that had been stored for 24 hours, 48 hours, 72 hours and 6 days. The changes in recipients' platelet levels are shown in Figure 1. The increase in circulating platelet levels and the persistence of platelets in the recipients' circulation were inversely related to the time of storage of the blood.

Dog 609 received 500r whole body radiation. Eleven days later, after thrombocytopenia had developed, 1,000 ml. of fresh blood collected in

[3] Kindly supplied by Dr. W. E. Mattison, Jr.

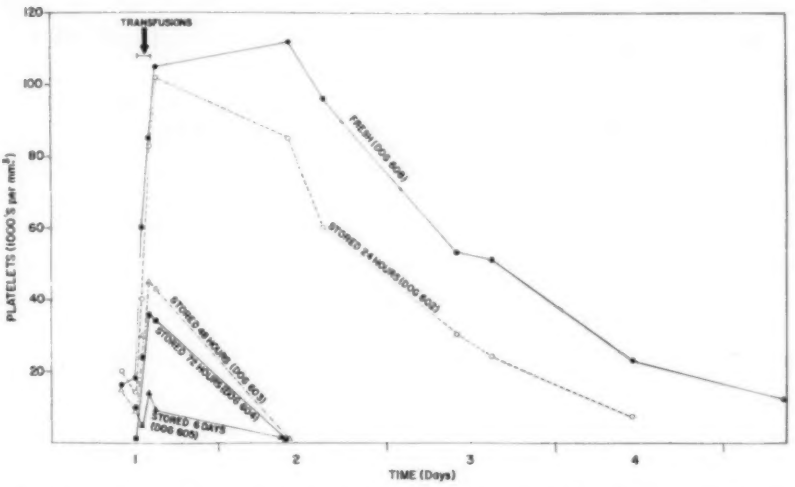

FIG. 1. Platelet levels following transfusion of 1,000 ml. fresh or stored blood using plastic bags containing ACD. All dogs had received 500r whole body radiation 8 to 11 days before transfusion.

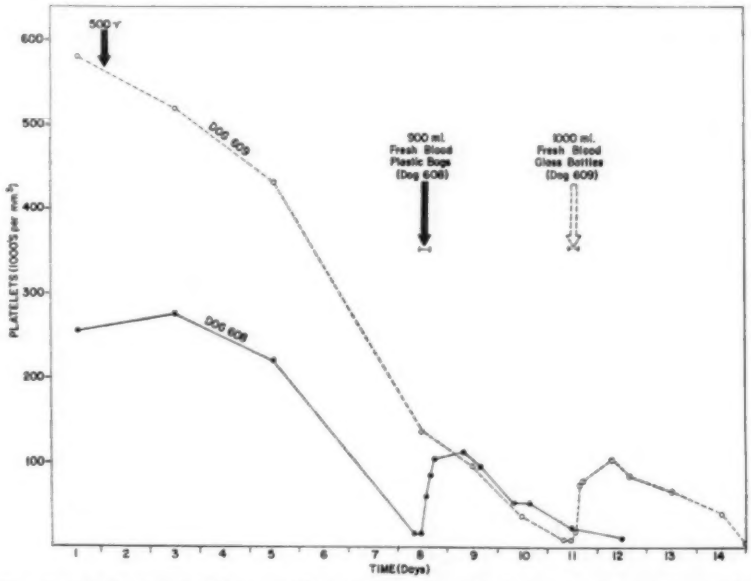
500 r
DOG 609
DOG 608
300 ml. Fresh Blood Plastic Bags (Dog 608)
1000 ml. Fresh Blood Glass Bottles (Dog 609)
PLATELETS (1000'S per mm³)
TIME (Days)

FIG. 2. Platelet levels following 500r whole body radiation and subsequent transfusion of fresh blood collected in plastic bags or glass bottles containing ACD.

TABLE I

Effects of Transfusion of 1,000 ml. of Fresh or Stored Blood on Platelet Levels of Irradiated, Thrombocytopenic Dogs

Dog Number	Weight (kg.)	Estimated Blood Volume (ml.)	Type of Container	Period of Storage	Platelets in Donor Blood† (per cu. mm.)	Calculated Platelet Rise (per cu. mm.)	Observed Platelet Rise	
							per cu. mm.	Per Cent of Calculated Rise
608*	7.7	690	Plastic bags	<1 hour	200,000	120,000	100,000	83
602	7.3	660	Plastic bags	24 hours	180,000	110,000	90,000	82
603	14.1	1,270	Plastic bags	48 hours	160,000‡	70,000	35,000	50
604	15.9	1,430	Plastic bags	72 hours	165,000	70,000	35,000	50
605	11.8	1,060	Plastic bags	6 days	255,000	125,000	<5,000	0
609	9.1	820	Glass bottles	<1 hour	220,000	120,000	100,000	83

* Dog 608 received 900 ml.

† Counts performed on day of donation.

‡ Counts performed on day of transfusion.

uncoated, glass vacuum bottles were administered. Figure 2 compares the changes in this recipient's platelet level with those observed in Dog 608 which received fresh blood collected in plastic bags. The platelet level increased by 100,000 per cu. mm. in each recipient, and the subsequent rates of disappearance of platelets from the circulation were similar. Also, the rates of disappearance of these transfused platelets were similar to the rates of disappearance of the animals' own platelets following irradiation.

In Table I, the observed platelet increases are compared with the calculated platelet increases in the dogs that received transfusions without phlebotomy. For purposes of comparison, the calculated platelet rise was estimated by assuming that if the platelets circulated, they would be distributed, immediately after transfusion, in a volume equal to the recipient's original blood volume plus the amount of blood infused. Infusions of fresh blood collected in glass as well as plastic containers and blood stored for 24 hours in plastic containers raised the platelet levels to >80 per cent of the calculated values. Infusions of blood stored for 48 to 72 hours raised the platelet levels to about 50 per cent of the calculated levels. Blood stored for 6 days did not significantly elevate the recipient's platelet level.

Dog 376 (16 kg.) and Dog 377 (16 kg.) were given 450r whole body radiation. Ten days later Dog 377 was exchange transfused with 2,675 ml. of blood that had been collected from 5 donor dogs into plastic bags and stored for 10 to 13 days. Platelet counts of the donor dogs on the day of

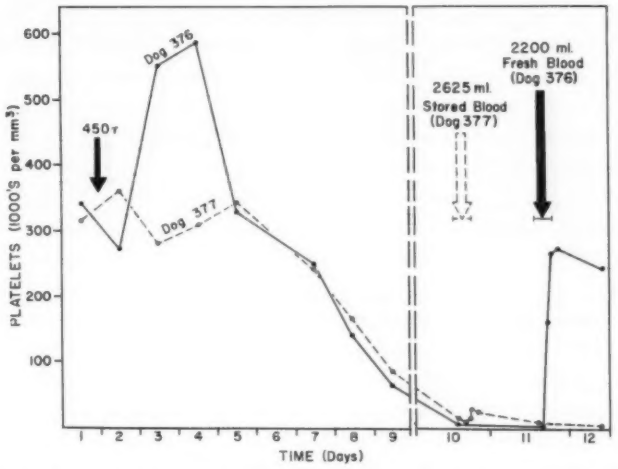

FIG. 3. Platelet levels following 450r whole body radiation and subsequent exchange transfusion of fresh or stored blood collected in plastic bags containing ACD.

donation ranged between 243,000 and 632,000 per cu. mm. On the day of transfusion, platelet counts on the stored blood from the 5 plastic bags ranged from 150,000 to 313,000 per cu. mm., but moderate clumping of the platelets was noted in the counting chambers. Before transfusion the recipient's platelet count was 10,000 per cu. mm. and immediately following exchange transfusion the count was 29,000 per cu. mm. Twenty-four hours later the platelet count was <1,000 per cu. mm. (Fig. 3). On the eleventh post-irradiation day, Dog 376 was exchange transfused with 2,200 ml. of fresh blood that had been collected in plastic bags. Platelet counts of the donor dogs just prior to phlebotomy ranged between 388,000 and 615,000 per cu. mm. This recipient's circulating platelet level increased from <1,000 to 273,000 per cu. mm. following transfusion. Twenty-four hours later the platelet level was 249,000 per cu. mm. (Fig. 3). Studies of blood coagulation were performed on each of these recipients before and 15 minutes after completion of transfusion. Results are shown in Table II.

Dog 610 (19 kg.) was given 10.0 ml. of rabbit anti-dog platelet serum intravenously. Striking thrombocytopenia occurred immediately (Fig. 4). Sixteen hours later the platelet count was <10,000 per cu. mm. A cross-transfusion between this animal and a compatible, normal donor dog was then performed until approximately 2,800 ml. of blood had been exchanged. Results are shown in Figure 4. There was a prompt rise in the thrombocytopenic recipient's (Dog 610) platelet level and a concomitant fall in the platelet level of the normal donor.

TABLE II

Effects of Exchange Transfusion of Fresh or Stored Blood on Blood Coagulation of Irradiated, Thrombocytopenic Dogs

		Platelets (per cu. mm.)	Whole Blood Clotting Time (minutes)*	Prothrombin Consumption (1-stage)	Clot Retraction (per cent)†	Plasma Fibrinogen (mg. per 100 ml.)	Clot Lysis
Dog 377	Pre-transfusion	10,000	23	Impaired	29	396	Negative
	Post-transfusion of stored blood	29,000	16	Low normal	57	224	Negative
Dog 376	Pre-transfusion	<1,000	49	Impaired	7	798	Negative
	Post-transfusion of fresh blood	273,000	19	Normal	82	316	Negative

* Normal range = 15–30 minutes

† Normal range = >70 per cent

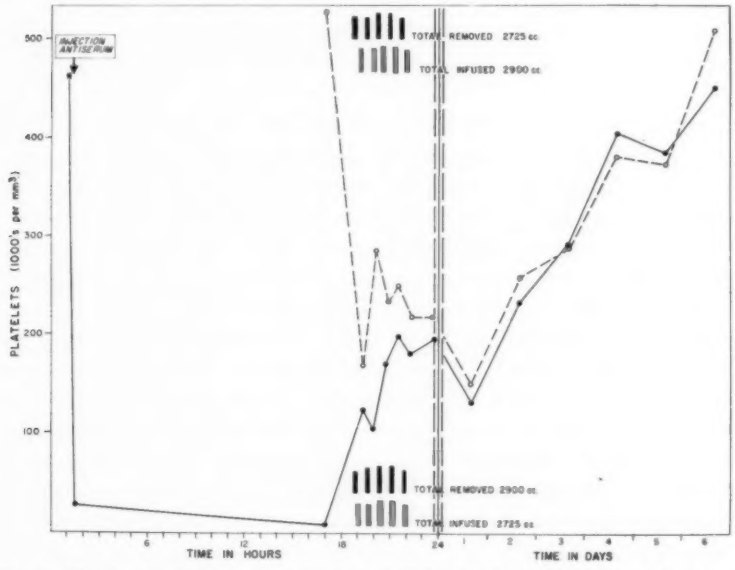

FIG. 4. Platelet levels following injection of 10.0 ml. of rabbit anti-dog platelet serum and subsequent cross-transfusion with a normal donor dog using plastic bags containing ACD.

DISCUSSION

In these studies, fresh blood transfused into irradiated, thrombocytopenic dogs elevated the platelet levels of the recipients. The slow rate of disappearance of the platelets from the recipients' circulation was evidence

of survival of the transfused platelets. Stored blood was less effective than fresh blood in elevating the platelet levels in thrombocytopenic recipients and the more rapid disappearance of the platelets from the circulation indicated that these transfused platelets survived less well.

Mustard (21), Gross and Staufenberg (22) and others have noted that blood stored for over one week may contain nearly normal numbers of platelets. The clot accelerating activity of platelets has been shown to be stable on storage for weeks (22) to months (11, 23). Hartmann and Conley (24) and others (11) demonstrated preservation of the clot retracting activity of platelets stored in plasma for 8 to 10 days, and Gross and Staufenberg (22) observed this function of the platelets in stored blood to be preserved for over 10 days. The present studies indicate, however, that platelets contained in blood stored for 6 days were not viable since infusion of such blood was ineffective in elevating platelet levels of thrombocytopenic dogs. Bell and colleagues (25) in studies of a thrombocytopenic human infant reported successful transfusion of platelets in fresh blood but transfusion of stored blood containing normal numbers of platelets was not followed by elevation of the recipient's platelet count. Thus, *in vitro* measurements of platelet numbers and of the clot accelerating and clot retracting functions of platelets are not adequate to determine platelet viability with respect to the ability of the platelets to survive when transfused into thrombocytopenic recipients.

Thrombocytopenia has been observed following transfusion of massive amounts of stored blood (26, 27). Post-transfusion survival of platelets was inversely related to the time of storage of the transfused blood (3, 27, 28, 29). The mechanism of the devlopment of thrombocytopenia following massive blood transfusions has been thought to be, in part, the deleterious effect of storage on the viability of the transfused platelets (3, 26 to 29). The present studies support this concept.

It has been reported (11, 30) that collection of blood into uncoated glass bottles results in significant loss of platelets during collection. Foaming of the blood during collection into vacuum bottles has been suggested as an additional factor causing platelet loss (11). Mustard and Walker (30), however, were unable to demonstrate any significant difference in *in vitro* platelet loss in blood collected in bottles by gravity or by use of a vacuum. It is generally agreed that platelets undergo marked alteration during blood coagulation and that contact with wettable surfaces accelerates the clotting of blood. In the present studies, transfusion of fresh blood collected into uncoated glass vacuum bottles was as effective as transfusion of fresh blood collected into plastic bags in elevating the platelet levels of irradiated thrombocytopenic dogs. Moreover, the subsequent rates of disappearance of the transfused platelets from the recipients' circulation were similar.

These results indicate that contact with glass *per se* does not render platelets non-viable.

Transfusion of fresh whole blood into irradiated dogs elevated the recipients' platelet counts to levels approaching the calculated maximum. The amounts of blood transfused were large (1,000 ml.) and caused pulmonary edema in 2 of the 5 recipients. The absolute platelet levels achieved were approximately 100,000 per cu. mm. Higher absolute platelet levels were achieved in the irradiated dog by exchange transfusions of larger amounts (2,200 ml.) of fresh blood. The absolute platelet level achieved by platelet transfusions depends upon a number of factors (3) including the number of viable platelets infused. Other techniques which provide large numbers of viable platelets include infusions of fresh blood from donors with thrombocytosis (31, 32), freshly prepared platelet-rich plasma (33 to 35) and freshly prepared platelet concentrates (33 to 35).

Cross-transfusion of approximately 2,700 ml. of blood between a normal dog and a dog rendered thrombocytopenic by an injection of rabbit anti-dog platelet serum resulted in a prompt rise of approximately 200,000 per cu. mm. in the thrombocytopenic dog's platelet count. There was a concomitant fall in the normal dog's platelet count presumably due to dilution with thrombocytopenic blood. Mattison (16) has shown that injection of rabbit anti-dog platelet serum resulted in thrombocytopenia that persisted for 2 to 5 days. The antibody could not be detected in the plasma of the dogs 15 minutes after injection of the serum (16). The present studies also indicate that the injected antibody does not persist in the circulation since it was possible to raise the platelet level of the thrombocytopenic recipient by transfusion and the infused platelets continued to circulate.

SUMMARY

Eight dogs were rendered thrombocytopenic by the administration of 450 to 500r whole body radiation. One dog was rendered thrombocytopenic by an injection of rabbit anti-dog platelet serum. After thrombocytopenia developed, compatible homologous blood was transfused either without phlebotomy, or by exchange transfusion, or by cross-transfusion. The viability of the platelets in the transfused blood was determined by observing the ability of the transfused platelets to survive in the thrombocytopenic recipients.

The platelets contained in fresh blood were viable as indicated by circulation of most of the transfused platelets and the subsequent slow disappearance of the platelets from the recipients' circulation. Survival of the platelets was not adversely affected by the use of uncoated glass containers.

The platelets contained in stored blood were less viable as indicated by initial survival of fewer of the transfused platelets and the subsequent more

rapid disappearance of the platelets from the recipients' circulation. Platelet viability was inversely related to the time of storage of the blood.

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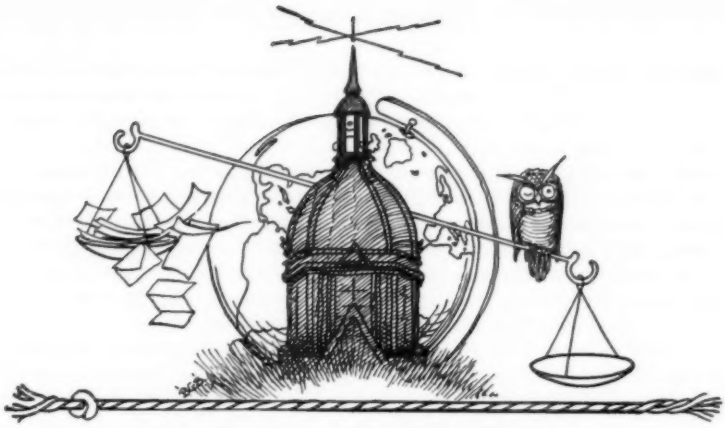

BOOK REVIEWS

Atlas of Exfoliative Cytology. Supplement 2 (Looseleaf). By GEORGE N. PAPANICOLAOU. 60 pp., $5.50. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1960.

The second supplement to the large *Atlas of Exfoliative Cytology* appearing originally in 1954, brings up to date the thinking in many fields of cytopathology. In addition, two new sections have been instituted with valuable material on each.

Series IP, on the vascular system, presents extremely timely and valuable material on circulating tumor cells in the blood. Series JP, entitled "Other Body Fluids", contains acceptable cellular representatives from cerebral spinal fluid.

Dr. Papanicolaou has not suffered from lack of material; in fact as he states, "sources of new material for the *Atlas* are practically inexhaustable". In this supplement his selection of illustrations and cases has been superb, and the representation on the color plates is sufficiently accurate to be of great value to the reader.

JOHN K. FROST

Neuropharmacology. Transactions of the Fifth Conference, May, 1959. Edited by HAROLD A. ABRAMSON. 251 pp., $6.00. The Josiah Macy, Jr. Foundation, New York, N. Y., 1960.

About half of this volume is devoted to recent work on the biochemistry and physiological function of amines which occur in the nervous system. The other half is concerned with electro-physiological aspects of the actions of drugs on the brain. Of particular interest are some experiments presented by K. F. Killam in which electrical correlates of learning and of the inhibition by drugs of the conditioned avoidance response were studied in cats by means of chronically-implanted electrodes. This conference appears to have been more successful than some of the earlier ones, in that criticism by the participants was generally at a higher level.

E. W. MAYNERT

Viral Infections of Infancy and Childhood. Symposium No. 10, Section on Microbiology, The New York Academy of Medicine. Edited by HARRY M. ROSE. 244 pp., $8.00. Paul B. Hoeber, Inc., Medical Division of Harper & Bros., New York, N. Y., 1960.

This book gives the reader an up to date view of the wide range of problems concerning viral infections of infancy and childhood. It is composed of sixteen chapters by thirty-four distinguished contributors of various disciplines from the United States, England and the Netherlands.

The first two chapters are devoted to experimental evidence demonstrating some of the roles of ribonucleic acid in viral infections. Several authors emphasize the inclusion of ECHO, Coxsackie and poliomyelitis into a single family of human enteroviruses. The laboratory, clinical and epidemiological characteristics of the ECHO and Coxsackie viruses are presented by different authorities and under different conditions. The difficult problem of determining the causal relationship between the enteroviruses and illness is amply shown by a discussion of paralytic disease. The present state of knowledge concerning influenza vaccination and it's indications in children are summarized. Viral interference is discussed along with the intriguing possibility of the utilization of interferon in the prevention of viral infections. Viral agents from the respiratory tract of infants and children are elaborated upon in some detail. The similarities of varicella and herpes zoster viruses along with the opportunity of studying viral latency is emphasized. The possibility of a measles vaccine is discussed. Confirmatory evidence concerning the efficacy of gamma globulin in infectious hepatitis and the excretion of the virus during the incubation period is presented.

This symposium depicts the mass amount of information that has accumulated about viral infections since the advent of tissue culture techniques. The question is just how much of this material is applicable to the clinician. Certainly, further epidemiological studies utilizing a more general population under variable circumstances together with the modern viral techniques are needed prior to making any firm conclusions concerning the causal, precipitating or predisposing influences of many of these viral agents. The theory of multi-causation will probably be as applicable to the viral infections as it is to other states of health.

As with any symposium there is some repetition of material, however, this is usually done in such a way as to give a different insight into the problems. There are numerous illustrations, charts, graphs and tables which are eloquently utilized by the authors. There is slight controversy among the authors concerning some of the material as illustrated by the recommendation of a widespread adoption of an influenza vaccination program by Davenport, but a more conservative approach by Curnen.

In summary this symposium presents new ideas, concepts and information pertaining to viral infections in the pediatric age group which would be of interest to the microbiologist, pediatrician, epidemiologist and public health worker.

J. L. RHYNE

Edema. Mechanisms and Management. A Hahnemann Symposium on Salt and Water Retention. Edited by JOHN H. MOYER AND MORTON FUCHS. 833 pp., $20.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

The size and comprehensive nature of such an edition as the Hahnemann Symposium on Edema make an objective appraisal difficult. One is smothered into stunned praise by size alone, or into condemnation by price or by the faults inherent in such an undertaking. The editors have included papers on essentially all aspects of salt and water physiology, pathology and therapeutics; the contents certainly transcend the title.

The volume has considerable duplication, particularly with respect to diuretics, their pharmacology and mechanisms of action. This in turn leads to inequalities of presentation so that some broad areas critical to the subject are relatively briefly presented

(e.g. renal tubular mechanisms of electrolyte control). There is a modest amount of contradiction between the various contributors, but this is reasonably structured into the framework of variations of opinion and interpretation of available data.

The symposium, however, offers many advantages which easily justify its preparation and recommend its perusal. It offers an agglomeration of material in this proliferative and somewhat confused field which can be found in no other single volume at this time. Reasonable correlative and summative efforts are made in the panel discussions wherein the line between proven and presumed fact is at least tentatively drawn. There is extensive bibliographical material for more detailed searching in the crannies of research publications. For the more practical minded, the volume contains informative appraisals of the many diuretic agents which have (with apologies for the expression) flooded the pharmacopoeia in recent years.

The Symposium on Edema would thus seem to be of positive value to the medical student for its physiological and pharmacologic data, to the teaching physician for its review of the rapidly advancing concepts in this field, and to the practitioner for assembled information on pharmacologic treatment of edematous states. And to all it offers a sobering assessment of their summated knowledge in the field of salt and water physiology.

JAMES C. ALLEN

Clinical Management of Behavior Disorders in Children, 2nd edition. By HARRY BAKWIN AND RUTH MORRIS BAKWIN. 597 pp., $11.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

The appearance of a second edition of the Bakwin Textbook attests to its favorable reception by physicians and to its ability to fulfill a useful role. When the first edition appeared this reviewer commented "this excellent text can be commended to all workers interested in the psychology and psychopathology of childhood." This new edition can be recommended in the same terms. It is about 100 pages longer; its bibliography has been amplified and made current; its discussion of certain issues has been expanded.

The authors, in an understandable effort to interest the pediatrician in taking the responsibility for management of childhood behavior disorders, are inclined to underestimate the difficulty in planning treatment programs for certain types of disorders. They do not, frequently enough, indicate that certain problems are more appropriately referred to the psychiatrist. For example, I would doubt seriously the competence of most pediatricians to undertake the treatment of childhood schizophrenia, homosexuality and juvenile delinquency.

While the orientation of the authors can be best described as eclectic, they are more inclined to emphasize organic factors than the present reviewer; the evidence for a somatic etiology for enuresis, homosexuality and sub-cultural mental retardation is less clear than the authors imply. However, such disagreements are to be expected in the present era where many of these issues are still quite unsettled.

In general, then, this text fills a useful role and constitutes a worthwhile addition to the bookshelf of any physician engaged in the care of children.

LEON EISENBERG

Metal Binding in Medicine. Proceedings of a Symposium sponsored by Hahnemann Medical College and Hospital, Philadelphia. Edited by MARVIN J. SEVEN AND L. AUDREY JOHNSON. 400 pp., $13.75. J. B. Lippincott Co., Philadelphia, Pa., 1960.

To save the time of the reader the first comment in this review must contain the fact

that this book is chiefly for someone specializing in an aspect of metal-binding: perhaps it may be used occasionally as a reference book by others. This is because the subject itself is highly specialized for one thing. Secondly the book is a symposium consisting of chapters written by many different authors, each one emphasizing his own detailed point of view and interest. Thirdly, its arrangement is such that an individual unfamiliar with the field cannot learn about the total subject nor even parts of it in an organized fashion.

So this is a book for the specialist, but even he is likely to be interested in only one or two chapters. The range of subjects covered is so tremendous and much of the material is so speculative that there are few indeed who would want to cover much of the field. This seems to be a rather standard kind of book following a symposium these days. The book is fairly large, covers a multitude of topics in a cross-sectional way, is necessarily disorganized and repetitive, covers many fringe areas that are not properly in the field of chelation, moves between great generalities and fine details of experiments, and is used here and there as a publication medium for laboratory results.

Here is an example of a book adding to the plethora of writing and talking that goes on in our society today. It would seem that someone thinks of a subject for a symposium which sounds new and exciting. Various kinds of support can be found for such symposia on the grounds of prestige, or of scientific honor for an institution, or as a supposed way to advance science. The idea gathers momentum, everybody gets prestige out of it, the organizers, the contributors, the supporters, and the publishers of the discussions. It seems to make no difference whether the subject has been covered in several different ways recently, often by the same contributors in as many different cities. A new excuse for a symposium has been found, another opportunity has been given for people to travel, and another excuse has been found to avoid doing some good, quiet, basic work. We can only hope that the Russians will emulate us and so not get too far ahead of us scientifically.

I do not wish to bring criticism on the heads of those who conjured up this particular symposium nor upon the contributors or the publishers. Actually this sort of thing is in the air today and the criticism is to be directed toward our scientific society in general, perhaps against our entire status-seeking behavior. The book required much effort and will look decorative on any shelf, but is otherwise of limited value.

FREDERICK W. BARNES, JR.

Communicable and Infectious Diseases. Diagnosis, Prevention and Treatment, 4th edition. By FRANKLIN H. TOP and collaborators. 812 pp., $20.00. The C. V. Mosby Co., St. Louis, Mo., 1960.

Two kinds of general texts on communicable disease might be justifiable. On one hand, a synopsis or handbook of the subject would be useful for quick reference and as a guide to further study. On the other hand, a fairly comprehensive reference text would also fulfill a useful purpose. This book aims somewhere between and fails to fulfill either need.

Perhaps the most serious shortcomings of this book are those of omission. It may be pardonable not to mention the relatively rare rat-bite fever in a section devoted to diseases secondary to mammalian bites. But for a text of this kind, not to mention at all such diseases as toxoplasmosis, cytomegalic inclusion body disease, and Trichomonas vaginitis seems incredible. Herpes simplex is noted only as a complication of purulent meningitis. One sentence each is devoted to monilial infection and roseola infantum. Only half a page is devoted to H. influenzae.

Since it is written from the clinical point of view, there is proportionally less information of a basic microbiological nature. While this is a permissable approach, the reader deserves in return a more knowledgeable and elegant presentation of the clinical aspects of the subject than is given here. For instance, staphylococcal pneumonia is discussed without the use of the term "pneumatocele".

The volume begins with a section on general considerations which is disappointing. It fails particularly to describe present concepts on non-specific immunity of both a cellular and humoral nature. Hypersensitivity is not well covered. The remainder of the book is devoted to communicable diseases grouped as to portal of entry. This rather odd organization, while confusing, is not itself a serious fault.

Individual chapters are praiseworthy. Commendable new sections on acute respiratory disease, the Echo and Coxsackie viruses, and pathogenic E. coli diarrhea have been added since the last edition. The chapter on diphtheria and histoplasmosis are particularly good. The illustrations in general are excellent. Despite these favorable features, it is doubtful that this book will take an important place in the literature on infectious diseases.

EDWARD J. OTTENHEIMER, JR.

Anatomy. A Regional Study of Human Structure. By ERNEST GARDNER, DONALD J. GRAY AND RONAN O'RAHILLY. 999 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa., 1960.

This is another of the growing number of shortened textbooks designed primarily for use in the drastically curtailed gross anatomy courses now in vogue in this country. Actually, however, when compared with the current editions of the three standard anatomical texts—Cunningham, Gray (American edition), Morris—it is not as greatly abridged as it appears at a first glance. For the three standard texts traditionally continue to include lengthy special chapters devoted to embryology and neuroanatomy. These have been omitted by Gardner *et al.* on the grounds that these subjects generally now are dealt with in separate courses for which special textbooks are available. However, relevant embryological as well as comparative-anatomical and functional data are presented at appropriate places throughout the book. If the special embryological and neuroanatomical chapters are eliminated from consideration, the present volume—basing my comparison on a rough estimate of the number of words in the text—is about 75 per cent the size of Gray, approximately 70 per cent as large as Cunningham, and slightly over 50 per cent the length of Morris.

The presentation of the subject matter is more regional than in the three standard texts, in which, indeed, the arrangement is wholly systematic. This book is not a truly regional anatomy, however; rather, to quote its authors, its position is one of "moderate regionalism," for the systematic presentation still prevails regionally. The general arrangement, however, should help the beginning student, particularly when reviewing a given part of the body.

Many students will be stimulated by the more than 1700 footnotes, which supplement the text by giving additional data and pertinent references to the literature, and by the well-selected lists of references appended to many chapters. They also will be particularly grateful for the excellent glossary of eponyms when they reach their clinical years.

One of the most difficult problems in teaching gross anatomy is to have the student realize that departures from textbook man are not necessarily abnormal, and that there is a considerable range of normal morphological variability. Shorter anatomical texts,

limited as they must be in scope, fail notably in this respect. The present book should be more successful, even though most of the data respecting variability are in the form of footnotes. I cannot help but wonder, however, whether the section entitled "Factors responsible for variations [in development and growth]" (pp. 90–92) may not defeat its intended purpose; for it seems likely to convey the impression to the beginning student that there is no such thing as truly *normal* biological variability.

The illustrations are less satisfactory than the text. All black-and-white, they number 658 (including 65 half-tone plates). Many are lacking in clarity and contrast. This is also true of some of the radiographic plates.

All things considered, this book represents a welcome addition to the list of gross anatomy texts. Although it is extremely unlikely that it will completely displace the larger, standard texts, even in the shorter courses, it should prove to be of considerable value. For those who wish to review their gross anatomy, it will be particularly useful.

WILLIAM L. STRAUS, JR.

Encyclopedia of Medical Syndromes. By ROBERT H. DURHAM. Foreword by T. R. Harrison. 628 pp., $13.50. Paul B. Hoeber, Inc., Medical Book Dept. of Harper & Bros. New York, N. Y., 1960.

Syndromes are the "concurrence or running together of constant patterns of abnormal signs or symptoms." These represent the careful observations of many physicians, largely since the eighteenth century, who fully described the experiments of nature presented to them. In the years following such descriptions many syndromes have received a basis in sound scientific principals; others remain associations of tantalizing interest.

Dr. Durham has assembled a scholarly volume culled from a hopelessly large literature and has included only those syndromes which are widely accepted or are adequately described and documented. Eponyms appear frequently and due allowance of each country's preference for eponyms chosen from their own citizens is given by study of the European literature. A representative entry includes all possible synonyms and a simple definition. The frequency of occurrence is estimated and a review of the pathogenesis, physiology and current views on the meaning is presented. A current reference is included chosen to provide the most insight into the meaning of the association. The changing nature of some syndromes as the etiology becomes known is adequately emphasized. An ample index is present.

Despite my initial aversion to such a rich collection of eponyms, I found myself leafing avidly into this kaleidoscope of new associations. This volume will provide a single authoritative source for information and references of approximately 1,000 syndromes. It thus fills a gap among present reference sources and should be included in all teaching and reference medical libraries. The quality of the production is satisfactory. Some of the descriptions would be strengthened by illustrations and some by original references.

FRANK IBER

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American Pharmacy, 5th edition. Edited by JOSEPH B. SPROWLS. 493 pp., $10.75. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Cardiac Emergencies and Related Disorders. Their Mechanism, Recognition and Management. By HAROLD D. LEVINE. 381 pp., $12.00. Landsberger Medical Books, New York, N. Y., 1960.

Cellular Aspects of Immunity. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. 495 pp., $10.50. Little, Brown & Co., Boston, Mass., 1960.

The Delayed Effects of Whole-Body Radiation: A Symposium. Jointly sponsored by the Operations Research Office and Walter Reed Army Institute of Research. Edited by Bernard B. Watson. 80 pp., $4.50. Published for Operations Research Office, The Johns Hopkins University by The Johns Hopkins Press, Baltimore, Md., 1960.

Demonstrations of Physical Signs in Clinical Surgery, 13th edition. By Hamilton Bailey. 928 pp., $14.50. John Wright & Sons, Bristol, England. The Williams & Wilkins Co., Baltimore, Md., 1960, exclusive U. S. agents.

Diabetic Care in Pictures, 3rd edition. Simplified Statements with Illustrations Prepared for the Use of the Patient. By Helen Rosenthal and Joseph Rosentahl. 237 pp., $4.50. J. B. Lippincott Co., Philadelphia, Pa., 1960.

Essentials of Pharmacology, 4th edition. By Frances K. Oldham, F. E. Kelsey and E. M. K. Geiling. 418 pp., $7.75. The J. B. Lippincott Co., Philadelphia, Pa., 1960.

French's Index of Differential Diagnosis. Edited by A. H. Douthwaite. 1111 pp., $24.00. The Williams & Wilkins Co., Baltimore, Md., 1960.

Meaning and Methods of Diagnosis of Clinical Psychiatry. By Thomas A. Loftus. 169 pp., $5.00. Lea & Febiger, Philadelphia, Pa., 1960.

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INDEX TO VOLUME 107

Pagination according to months:

July, 1960, 1–62
August, 1960, 63–132
September, 1960, 133–198
October, 1960, 199–245
November, 1960, 247–295
December, 1960, 297–371

Acid-Base Physiology . . . 247
Amyloid. Fluorescent Stain for . . . 320
Antimicrobial Drugs. In Vitro and In Vivo Susceptibility of Proteus Species to the Action of Certain . . . 337

Bacterial Pyrogen. The Effect of Intraarticular Pressure on Pathogenesis of Fever in Joint Disease . . . 22
Bahnson, Henry T.: (see Jabaley, Michael E.) . . . 126
Bang, Frederik B.: (see McGowan, Thomas R.) . . . 63
Bard, Philip: Thyroid Activity During Hypothermia Produced Without the Use of Drugs . . . 163
Berger, Elwin: Hormonal and Ionic Influence on Uterine Activity . . . 125
Bergman, Ronald A.: Further Observations on the Localization of Glycogen in Frog Striated Muscle . . . 307
Blalock, Alfred (see Isaacs, James P.) . . . 105
Blood Pressure Changes Conditioned to Painful Stimuli (Schizokinesis) . . . 72
Bodian, David: Inhibitory Effects of Passive Antibody on Virulent Poliovirus Excretion and on Immune Response in Chimpanzees . . . 143
Boitnott, John K.: (see Oppenheimer, Ella H.) . . . 297
Book Reviews . . . 60, 129, 197, 241, 291, 359
Brummer, Donald L.: (see Said, Sami I.) . . . 255

Carter, Darryl: Myocardial Metabolism During Coronary Perfusion with Gaseous Oxygen . . . 125
Catecholamine and 17-Hydroxycorticosteroid Output in Dogs with Transplanted Adrenal Glands. Isaacs, James P., Blalock, Alfred and Migeon, Claude J. . . . 105
Cats. Thyroid Activity . . . 163
Clot Retraction. Components of Blood Necessary for . . . 90
Cluff, Leighton E.: (see Lewis, George W.) . . . 22
(see Reynolds, Richard C.) . . . 278
Colistin in the Treatment of Infections of the Urinary Tract. The Use of . . . 133
Collaborative Cerebral Palsy Study. Hardy, Janet . . . 57
Colloid Milium. An Easy Histological Differentiation Between Cutaneous Amyloidosis and Senile Elastosis . . . 320
Components of Blood Necessary for Clot Retraction. Corn, Milton, Jackson, Dudley P. and Conley, C. Lockard . . . 90
Congenital Arteriovenous Fistula in the Neck—48 Year Follow-Up of a Patient Operated upon by Dr. Halsted. Ravitch, Mark M. and Gaertner, Robert A. . . . 31
Conley, C. Lockard: (see Corn, Milton) . . . 90
Conti, Charles R. Jr.: Studies on the Pathogenesis of Staphylococcal Infections . . . 127
Corn, Milton: Components of Blood Necessary for Clot Retraction . . . 90
Cortico-Steroid Therapy, Adrenal. Pancreatitis in Children Following . . . 297

Decerebrate Cats. Chronic Experiments on . . . 163
Diffusion, Pulmonary. Shunting Effect of Extreme Impairment of . . . 255

Drugs, Antimicrobial. In Vitro and In Vivo Susceptibility of Proteus Species to the Action of Certain ... 337
Dykman, Roscoe A.: Experimental Psychogenic Hypertension: Blood Pressure Changes Conditioned to Painful Stimuli (Schizokinesis) ... 72

Eastman, Nicholson J.: The Obstetric Etiology of 500 Cases of Cerebral Palsy in the New York Area ... 58
Elastosis, Senile. An Easy Histological Differentiation Between Cutaneous Amyloidosis, Colloid Milium and ... 320
Experimental Psychogenic Hypertension: Blood Pressure Changes Conditioned to Painful Stimuli (Schizokinesis) Dykman, Roscoe A. and Gantt, W. Horsley ... 72

Familial Occurrence of Hypoplastic Right Lung with Systemic Arterial Supply and Venous Drainage "Scimitar Syndrome". Neill, Catherine A., Ferencz, Charlotte, Sabiston, David A. and Sheldon, Huntington ... 1
Ferencz, Charlotte: (see Neill, Catherine A.) ... 1
Fetal Skin for the Study of Skin Diseases. Organ Cultures of ... 63
Fever in Joint Disease. Pathogenesis of ... 22
Fistula in the Neck, Congenital Arteriovenous ... 31
Fluorescent Stain for Amyloid. An Easy Histological Differentiation Between Cutaneous Amyloidosis, Colloid Milium and Senile Elastosis. Kurban, Amal K. ... 320
Further Observations on the Localization of Glycogen in Frog Striated Muscle. Bergman, Ronald A. ... 307

Gaertner, Robert A.: (see Ravitch, Mark M.) ... 31
Gamble, J. L. Jr.: Sodium and Chloride and Acid-Base Physiology ... 247
Gantt, W. Horsley: (see Dykman, Roscoe A.) ... 72
Geary, Paul, Jr.: (see Jabaley, Michael E.) ... 126
Glancy, David L.: (see Jabaley, Michael E.) ... 126
Glycogen in the Myocardium. Heart Failure Associated with Unusual Deposition of ... 307
in Frog Striated Muscle. Further Observations on the Localization of ... 307

Hardy, Janet: The Collaborative Cerebral Palsy Study ... 57
Heart Failure Associated with Unusual Deposition of Glycogen in the Myocardium. Mehrizi, Ali and Oppenheimer, Ella H. ... 329
Hook, Edward W.: (see Ribble, John C.) ... 108
(see Petersdorf, Robert G.) ... 133
In Vitro and In Vivo Susceptibility of Proteus Species to the Action of Certain Antimicrobial Drugs ... 337
Hormonal and Ionic Influences on Uterine Activity. Berger, Elwin ... 125
Humphries, J. O'Neal: Occurrence of Hypertensive Toxemia of Pregnancy in Mother-Daughter Pairs ... 271
Hyde, H. van Zile: The Physician as a World Citizen ... 199
17-Hydroxycorticosteroid Output in Dogs with Transplanted Adrenal Glands ... 105
Hyperglyceridemia and Lipemia Retinalis in Hypopituitarism. Tabatznik, Bernard and Rabinowitz, David ... 175
Hypertension, Experimental Psychogenic ... 72
Hypopituitarism ... 175
Hypoplastic Right Lung with Systemic Arterial Supply ... 1
Hypothermia Produced Without the Use of Drugs. Thyroid Activity During ... 163

Inactivation of T2+ Bacteriophage by Lysozyme. Ribble, John C. and Hook, Edward W. ... 108
Infections of the Urinary Tract. The Use of Colistin in the Treatment of ... 133

Inhibitory Effects of Passive Antibody on Virulent Poliovirus Excretion and on Immune Response in Chimpanzees. Bodian, David and Nathanson, Neal 143
Interaction of Serum and Sodium Salicylate: Changes During Acute Infection and Its Influence on Pharmacological Activity. Reynolds, Richard C. and Cluff, Leighton E. .. 278
In Vitro and In Vivo Susceptibility of Proteus Species to the Action of Certain Antimicrobial Drugs. Hook, Edward W. and Petersdorf, Robert G. 337
Isaacs, James P.: Catecholamine and 17-Hydroxycorticosteroid Output in Dogs with Transplanted Adrenal Glands 105

Jabaley, Michael E.: Studies with Heparin and Protamine in Extracorporeal Circulation .. 126
Jackson, Dudley P.: Survival of Platelets Transfused into Thrombocytopenic Recipients .. 349
(see Corn, Milton) 90
Johns Hopkins Medical Society. Abstracts of Papers Presented Before the Meetings of .. 57, 125

Kaposi's Sarcoma in a Father and Son. Zeligman, Israel 208
Krevans, Julius R.: (see Jackson, Dudley P.) 349
Kurban, Amal K.: Fluorescent Stain for Amyloid. An Easy Histological Differentiation Between Cutaneous Amyloidosis, Colloid Milium and Senile Elastosis 320

Lewis, George W.: Pathogenesis of Fever in Joint Disease. The Effect of Intraarticular Pressure and Intraarticular Bacterial Pyrogen 22
Lipemia Retinalis in Hypopituitarism 175
Lysozyme. Inactivation of T2+ Bacteriophage by 108

McGowan, Thomas R.: Organ Cultures of Human Fetal Skin for the Study of Skin Diseases 63
McKusick, Victor A.: (see Manley, Keith A.) 213
Manley, Keith A.: Serum Cholesterol Values in a Hospital Population 213
Mehrizi, Ali: Heart Failure Associated with Unusual Deposition of Glycogen in the Myocardium 262
Migeon, Barbara Ruben: Some Observations on the Staphylococcus in a Pediatric Out-Patient Population 262
Migeon, Claude J.: (see Isaacs, James P.) 105
Minchew, B. Harvey: (see Migeon, Barbara Ruben) 262
Multiple Myeloma with Myelofibrosis and with Polycythemia Vera: Further Evidence of a Relationship Between the Myeloproliferative Disorders. Spickard, Anderson 234
Muscle. Further Observations on the Localization of Glycogen in Frog Striated 307
Myeloma, Multiple, with Myelofibrosis 234
Myeloproliferative Disorders 234
Myocardial Metabolism During Coronary Perfusion with Gaseous Oxygen. Carter, Darryl .. 125
Myocardium. Heart Failure Associated with Unusual Deposition of Glycogen in the 307

Nathanson, Neal: (see Bodian, David) 143
Neill, Catherine A.: The Familial Occurrence of Hypoplastic Right Lung with Systemic Arterial Supply and Venous Drainage "Scimitar Syndrome" 1
Neu, Harold C.: (see Randall, Harry G.) 127

Obstetric Etiology of 500 Cases of Cerebral Palsy in the New York Area. Eastman, Nicholson J. 58
Occurrence of Hypertensive Toxemia of Pregnancy in Mother-Daughter Pairs. Humphries, J. O'Neal 271
Oppenheimer, Ella H.: Pancreatitis in Children Following Adrenal Cortico-Steroid Therapy 297
(see Mehrizi, Ali) 329

Organ Cultures of Human Fetal Skin for the Study of Skin Diseases. McGowan, Thomas R. and Bang, Frederik B. ... 63

Pancreatitis in Children Following Adrenal Cortico-Steroid Therapy. Oppenheimer, Ella H. and Boitnott, John K. ... 297
Pathogenesis of Fever in Joint Disease. The Effect of Intraarticular Pressure and Intra-articular Bacterial Pyrogen. Lewis, George W. and Cluff, Leighton E. ... 22
Patterson, John L. Jr.: (see Said, Sami I.) ... 255
Petersdorf, Robert G.: The Use of Colistin in the Treatment of Infections of the Urinary Tract ... 133
(see Hook, Edward W.) ... 337
The Physician as a World Citizen. Hyde, H. van Zile ... 199
Platelets Transfused into Thrombocytopenic Recipients. Survival of ... 349
Poikilothermia ... 163
Polycythemia Vera ... 234
Porphyrin Uptake by Tumor Tissue Following Parenteral Administration. Quantitative Determination of ... 228
Proteus Species to the Action of Certain Antimicrobial Drugs. In Vitro and In Vivo Susceptibility of ... 337
Pulmonary Diffusion. Shunting Effect of Extreme Impairment of ... 255

Quantitative Determination of Porphyrin Uptake by Tumor Tissue Following Parenteral Administration. Winkelman, James and Rasmussen-Taxdal, David S. ... 228

Rabinowitz, David: (see Tabatznik, Bernard) ... 175
Randall, Harry G.: A Smooth Muscle Assay in the Study of Hypersensitivity ... 127
Rasmussen-Taxdal, David S.: (see Winkelman, James) ... 228
Ravitch, Mark M.: Congenital Arteriovenous Fistula in the Neck—48 Year Follow-Up of a Patient Operated upon by Dr. Halsted in 1911 ... 31
Relationship Between Potassium Binding and Oxidative Phosphorylation in Mitochondria. Scott, Robert L. ... 127
Reynolds, Richard C.: Interaction of Serum and Sodium Salicylate: Changes During Acute Infection and Its Influence on Pharmacological Activity ... 278
Ribble, John C.: Inactivation of T2+ Bacteriophage by Lysozyme ... 108

Sabiston, David A.: (see Neill, Catherine A.) ... 1
Said, Sami I.: Shunting Effect of Extreme Impairment of Pulmonary Diffusion ... 255
Scott, Robert L.: Relationship Between Potassium Binding and Oxidative Phosphorylation in Mitochondria ... 127
Serum and Sodium Salicylate, Interaction of ... 278
Serum Cholesterol Values in a Hospital Population. Manley, Keith A. and McKusick, Victor A. ... 213
Sheldon, Huntington: (see Neill, Catherine A.) ... 1
Shunting Effect of Extreme Impairment of Pulmonary Diffusion. Said, Sami I., Thompson, W. T. Jr., Patterson, John L. Jr. and Brummer, Donald L. ... 255
Skin Diseases. Organ Cultures of Human Fetal Skin for the Study of ... 63
Smooth Muscle Assay in the Study of Hypersensitivity. Randall, Harry G. and Neu, Harold C. ... 127
Sodium and Chloride and Acid-Base Physiology. Gamble, J. L. Jr. ... 247
Some Observations on the Staphylococcus in a Pediatric Out-Patient Population. Migeon, Barbara Ruben, and Minchew, B. Harvey ... 262
Spencer, Frank C.: (see Jabaley, Michael E.) ... 126
Spickard, Anderson: Multiple Myeloma with Myelofibrosis and with Polycythemia Vera: Further Evidence of a Relationship Between the Myeloproliferative Disorders ... 234

Staphylococcus in a Pediatric Out-Patient Population. Some Observations on the 262
Studies with Heparin and Protamine in Extracorporeal Circulation. Jabaley, Michael E., Geary, Paul, Jr., Glancy, David L., Taylor, Terry W., Tomkins, Ronald K., Spencer, Frank C. and Bahnson, Henry T. 126
on the Pathogenesis of Staphylococcal Infections. Conti, Charles R. Jr. 127
Survival of Platelets Transfused into Thrombocytopenic Recipients. Jackson, Dudley P. and Krevans, Julius R. 349
Systemic Arterial Supply and Venous Drainage "Scimitar Syndrome" 1

Tabatznik, Bernard: Hyperglyceridemia and Lipemia Retinalis in Hypopituitarism 175
Taylor, Terry W.: (see Jabaley, Michael E.) 126
Thompson, W. T. Jr.: (see Said, Sami I.) 255
Thrombocytopenic Recipients. Survival of Platelets Transfused into 349
Thyroid Activity During Hypothermia Produced Without the Use of Drugs. Bard, Philip and Woods, James W. 163
in Cats 163
During Cold Exposure 163
Tomkins, Ronald K.: (see Jabaley, Michael E.) 126
Toxemia of Pregnancy 271
Tumor Tissue Porphyrin Uptake Following Parenteral Administration 228

Urinary Tract. The Use of Colistin in the Treatment of Infections of the 133
Use of Colistin in the Treatment of Infections of the Urinary Tract. Petersdorf, Robert G. and Hook, Edward W. 133

Virulent Poliovirus Excretion. Inhibitory Effect of Passive Antibody on 143

Winkelman, James: Quantitative Determination of Porphyrin Uptake by Tumor Tissue Following Parenteral Administration 228
Woods, James W.: (see Bard, Philip) 163

Zeligman, Israel: Kaposi's Sarcoma in a Father and Son 208

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Further Observations on the Localization of Glycogen in Frog Striated Muscle. RONALD A. BERGMAN 307

Fluorescent Stain for Amyloid. An Easy Histological Differentiation Between Cutaneous Amyloidosis, Colloid Milium and Senile Elastosis. AMAL K. KURBAN 320

Heart Failure Associated with Unusual Deposition of Glycogen in the Myocardium. ALI MEHRIZI AND ELLA H. OPPENHEIMER 329

In Vitro and in Vivo Susceptibility of Proteus Species to the Action of Certain Antimicrobial Drugs. EDWARD W. HOOK AND ROBERT G. PETERSDORF 337

Survival of Platelets Transfused into Thrombocytopenic Recipients. DUDLEY P. JACKSON AND JULIUS R. KREVANS 349

Book Reviews 359

Index to Volume 107 367

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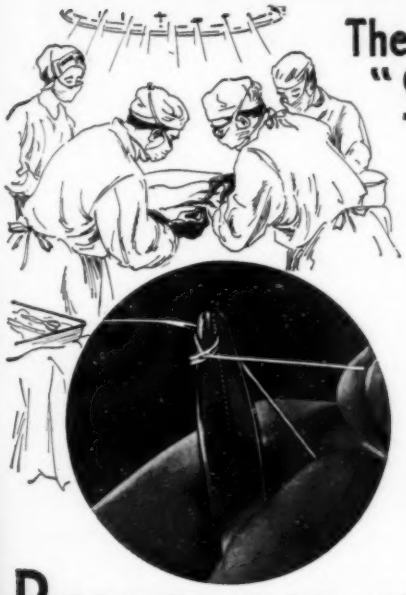

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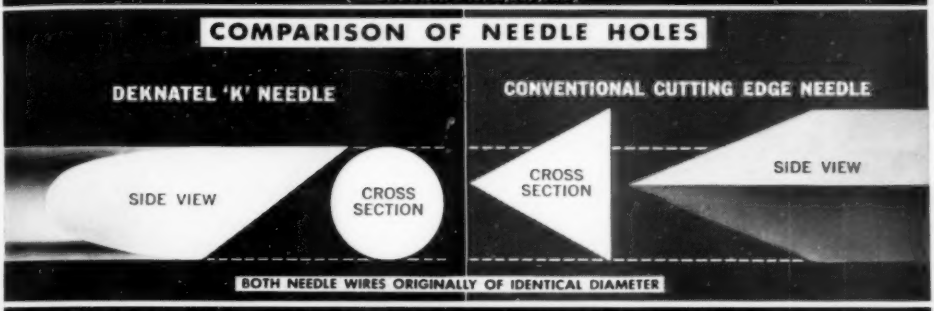

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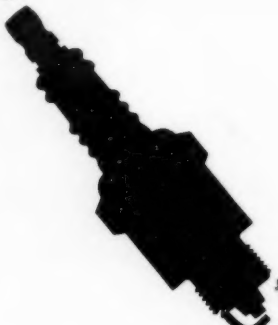

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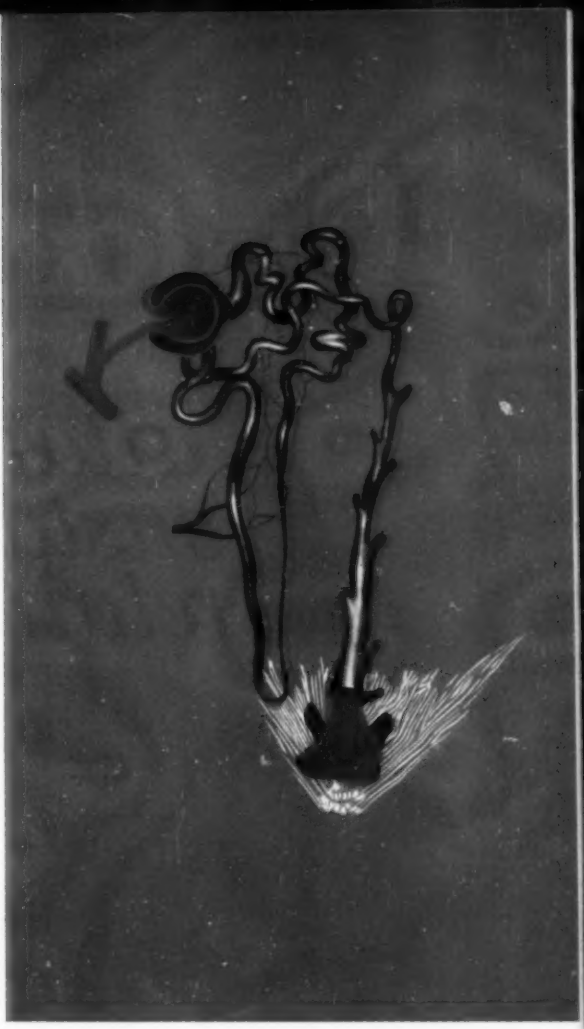

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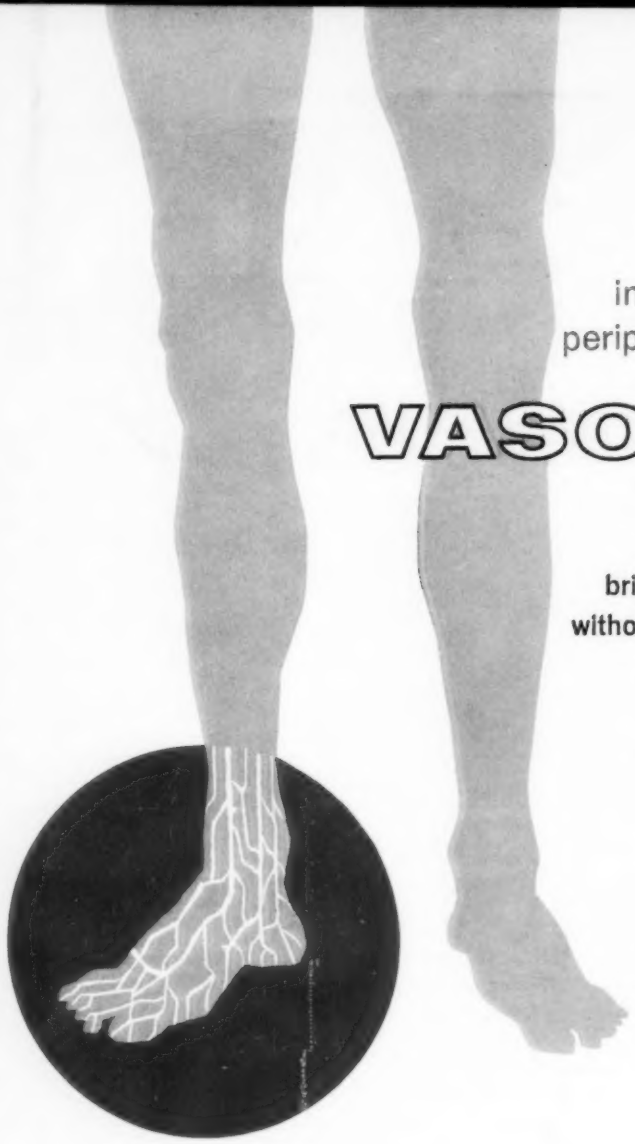

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